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## REVIEW ARTICLE

# Molluscs and echinoderms aquaculture: biological aspects, current status, technical progress and future perspectives for the most promising species in Italy

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## Abstract

Shellfish aquaculture is a widespread activity in the Italian peninsula. However, only two bivalve species are mainly cultured along the coastline of that country: the Mediterranean mussel *Mytilus galloprovincialis* and the Manila clam *Venerupis philippinarum* (*Ruditapes philippinarum*). By contrast, just a few other mollusc species of commercial interest are scarcely reared at a small-scale level.

After analysing the current status of Italian shellfish production, this paper reports and discusses the potential for culturing several different invertebrate species [*i.e.*, the European flat oyster *Ostrea edulis*, the grooved

carpet shell *Venerupis decussata* (*Ruditapes decussatus*), the razor clams *Ensis minor* and *Solen marginatus*, the cephalopod *Octopus vulgaris*, and the purple sea urchin *Paracentrotus lividus*] in this country.

In addition, a detailed overview of the progress made in aquacultural techniques for these species in the Mediterranean basin is presented, highlighting the most relevant bottlenecks and the way forward to shift from the experimental to the aquaculture phase. Finally, an outlook of the main economic and environmental benefits arising from these shellfish culture practices is also given.

## Introduction

### Current status of the Italian shellfish aquaculture

The Italian shellfish production amounted to 181,455 t in 2008 (FAO, 2011), corresponding to 68% of the total Italian aquaculture production and ranking Italy in the 3<sup>rd</sup> position in Europe, after France and Spain (189,070 and 185,153 t, respectively). Similarly to what happens to finfish production and unlike the two best shellfish producers in Europe, shellfish aquacultural practices are very scarcely diversified in Italy, since the only two species predominantly cultured are the Mediterranean mussel (*Mytilus galloprovincialis*; 123,010 t) and the Manila clam [*Venerupis philippinarum* (*Ruditapes philippinarum*); 58,445 t]. Only sporadic activities of rearing can be listed for other species, currently at a small-scale aquaculture level, as in the case of the oysters *Crassostrea gigas* and *Ostrea edulis*, and the grooved carpet shell *Venerupis decussata* (*Ruditapes decussatus*) or still as an experimental aquaculture example (*Modiolus barbatus*).

About molluscs culture in Italy, old traditions coexists with modern intensive farming techniques (Prioli, 2004). Mussel culture covers a total surface of about 20,000 ha and is realised by 263 companies that give employment to 1,400 people (Prioli, 2008). The cycle is based on the recruitment of wild spat largely available in many areas (namely, Apulia, Veneto and Emilia-Romagna regions) where the grow-out plants are located. The culture of *Mytilus galloprovincialis* is widely diffused along the coasts of the country (included those of Sardinia and Sicily, but not those of Basilicata, Calabria and Tuscany), where different kind of rearing techniques are applied. The traditional ones (fixed systems) are mostly located in

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sheltered coastal and in lagoon areas (gulf of Trieste, gulf of Taranto, Veneto lagoon, etc.), while the new long-lines rearing systems (single long-line *ventia* and Trieste or *multi ventia* long-line in open sea and in partially or fully sheltered areas, respectively) have been more and more diffused in offshore farms. The single *ventia* plants have been spread for 20 years and currently represent around 75% of total linear metres (2,000,000) of long-line quoted in Italy. The culture of the Manila clam started in Italy in the 1980s, when 200,000 juveniles from a North European hatchery were introduced in the Venice lagoon (southern basin) (Cesari and Pellizzato, 1985). Afterwards, this species was introduced in other areas of the Po river delta [Sacca di Goro (Ferrara), Sacca del Canarin-Porto Tolle (Rovigo) and Grado-Marano lagoon (Udine)].

Currently, the Venice lagoon is the most important production site for this bivalve, where 50% of the total Italian production is realised (Zentilin et al., 2008), followed by the area of the Po river delta in Emilia Romagna region (mainly Sacca di Goro) (28% of the total production) and in Veneto region (21%). The Grado-Marano lagoon very scarcely impacts on the total production (1%). Thanks to the optimal conditions found in North Adriatic areas,

this species spreads spontaneously and now the farmers move from rearing practices to management of production areas in a more or less controlled way. Manila clam culture covers a surface of about 940,000 ha and gives employment to 4000 to 5000 people (Turolla, 2008). Similarly to mussel culture, the spat is mainly collected from the wild (95% of the total utilised), but a hatchery seed production is also realised. Only two hatcheries (i.e., ALMAR and TURBOT, this last producing 10 to 50 million of clam seeds year<sup>-1</sup>) are currently operating in Italy, while in Europe a total of 34 mollusc hatcheries were recently listed (Robert, 2009).

In agreement with the EU Regulations [CE Reg. 2073/2005 (European Commission, 2005) and CE Reg. 853/2004 (European Commission, 2004)] for the marketing of shellfish, 125 centres for shellfish depuration and 320 centres for shellfish shipping (20 of which are located on boats in the service of equipment) are operational in Italy (Prioli, 2008).

A third cultured species is the grooved carpet shell *Venerupis decussata*, amounting to about 100 t in 2008 (FAO Fisheries and Aquaculture Department, 2011). The interest for the cultivation of that species has significantly increased in the last years due to its higher commercial value in comparison to that of the non-native species Manila clam. Also for oysters, despite their high market request, the amount produced is currently negligible, which puts Italy in the first position among the importer countries (with a stable quantity around 7400 t in the years 2006-2007: <http://www.globefish.org/oysters-may-2008.html>).

The main problems involving traditional shellfish culture are related to the seasonality of the production (for mussels mainly concentrated between May and September), the lack of traditional processes to obtain a product with a higher added value (currently the amount transferred to industry for processing is lower than 1% of total mussel production) (Prioli, 2004), the extensive seaweed blooms, and the algal blooms responsible for biotoxin risks. The lack of experimentation on the actual possibility to develop effective and economic systems for detoxification is a critical point for the future of this important aquaculture sector, due to serious economic losses during the closure of commercial shellfisheries caused by periodic harmful algal blooms. However, some results recently documented in the literature seem very promising about the possibility to detoxify the molluscs once contaminated (Marcaillou et al., 2010; Medhioub et al., 2010), or to prevent the contamination by innovative rearing systems (Serratore, 2011).

The perspectives of traditional cultures for

mussels currently are: i) a positive trend towards the settlement of new production sites along the coastline of those Italian regions offering more favourable conditions for mussel growing; ii) a marked interest for finding new simplified rearing techniques. Also in the case of the clam culture, some priorities can be listed for promoting further development, such as: i) an adequate management of the lagoons where this species is cultured. Indeed, during summer there are often anoxic crises and excessive growth of seaweed due to the peculiar condition of eutrophy; ii) a proper management of the nursery areas, appointed to the production of the seed which is mainly obtained (95%) from the wild (Turolla, 2008).

The high consumption of shellfish associated with the seasonality of the national production (mainly in the case of mussels) and the lack of diversification of production typical of the Italian shellfish aquaculture generate large volumes of imported products, as shown in Table 1. In 2011, the importing of mussels – ranking the first position among the seafood consumed in Italy – dramatically increased (58,300 t, +50% compared to 2010) (<http://www.globefish.org/bivalves-june-2012.html>), partly due to the current economic crisis that has pushed consumption towards products of lower commercial value.

To give impetus to the sector, some inter-

ventions on traditional species and a greater diversification of farmed species are essential. As far as the first aspect is concerned, appropriate business strategies need to be adopted in order to maintain the functional product price and to create new market niches through appropriate techniques of processing and of storage (freezing) of the product.

At a national and regional level, some research has recently been carried out to stimulate the culture of new species of molluscs. Most of the results are documented in grey literature and in final reports written in Italian language, and have had a very scarce impact on the international literature. Although much research has been carried out since the 1990s, no significant progress has been made in the Italian aquaculture landscape. As a matter of fact, for the cultured species it barely changed over the last 20 years. The main issues tackled in the studies of mollusc species which are deemed as promising for Italian aquaculture are summarised in Table 2.

In the following chapters, a detailed analysis of the most promising species for diversification of Italian shellfish culture is reported. Precisely, our attention will focus on some species that are supposed to change the landscape of Italian aquaculture in: i) the short term, as rearing techniques are already established (cf. the oyster *Ostrea edulis* and the

**Table 1. Quantity of different species of molluscs imported in Italy in 2008 (FAO, 2011).**

Commodity	Quantity, t
Frozen	
Squids ( <i>Ommastrephes sagittatus</i> , <i>Loligo</i> spp.)	65,518
Octopus	50,945
Squids ( <i>Illex</i> spp.)	21,399
Cuttlefishes	19,230
Squids nei <sup>o</sup>	8271
Cuttlefishes ( <i>Sepia officinalis</i> , <i>Rossia macrosoma</i> , <i>Sepiola rondeleti</i> )	163
Live, fresh or chilled <sup>o</sup> (prepared or preserved)	
Mussel meat (prepared or preserved, not in airtight containers)	9279
Molluscs nei (prepared or preserved)	7627
Oysters nei	5550
Cuttlefishes and squids nei	4755
Octopus	2764
Squids nei	2760
Squids ( <i>Ommastrephes sagittatus</i> , <i>Loligo</i> spp.)	1576
Other aquatic invertebrates (prepared or preserved)	1406
Cuttlefishes (other than live, fresh or chilled)	1026
European flat oyster (shucked or not)	740
Squids (other than live, fresh or chilled)	739
Octopus (dried, salted or in brine)	438
Mussel meat (prepared or preserved, in airtight containers)	373
Squids (dried, salted or in brine)	110
Total	204,669

<sup>o</sup>Not elsewhere identified, according to FAO. <sup>o</sup>Live, fresh or chilled, unless otherwise specified in brackets.

grooved carpet shell *Venerupis decussata*); or in ii) the medium-long term, as much research has been done or is going to develop the different phases of the breeding cycle (cf. the razor clams *Ensis minor* and *Solen marginatus*, and the common octopus *Octopus vulgaris*).

Lastly, the state of the art of the purple sea urchin *Paracentrotus lividus* is also given. Indeed, because of its significant marketing potential, the aquacultural activities of this species are undergoing a noteworthy improvement and its gonad quality is currently undertaken in many European regions.

## Perspectives of Italian shellfish aquaculture

### European flat oyster

#### Distribution, habitat and exploitation

The European flat oyster *Ostrea edulis* (Linnaeus, 1758) belongs to the Ostreidae family (Rafinesque, 1815) and is native of Europe. This species naturally lives in a region going from the Norwegian fjords to Morocco (North eastern Atlantic coasts) and in the Mediterranean sea (Jaziri, 1990) up to the Black sea coasts (Alcaraz and Dominguez, 1985). It is also found in South Africa, North-eastern America (from Maine to Rhode Island), Canada, Nova Scotia, New Brunswick

and British Columbia, probably imported from population whose ancestors were from the Netherlands (Vercaemer *et al.*, 2006).

*Ostrea edulis* is a typical filter feeder, filtering phytoplankton, copepod larvae, protozoans and detritus as food. Being a sessile organism, it lives fixed to a hard substrate and its feeding entirely depends on the resources naturally present in the surrounding environment. As a matter of fact, food is pumped in with the seawater and removed by the gills (Laing *et al.*, 2005), filtering even up to 25 L h<sup>-1</sup> depending on animal size and temperature (Korringa, 1952). This species is typical of coastland, estuarine and marine environments and sheltered areas, preferring hard substrates as rocks or artificial structures but also muddy sand, muddy gravel with shells, and hard silt. It lives in brackish and marine seawater, having an optimum of salinity rounding between 17 and 26 PSU (Blanco *et al.*, 1951) up to 40 metres deep.

*Ostrea edulis* is similar to other species of oysters widely cultivated in many regions of the world, like the Pacific cupped oyster *Crassostrea gigas* (Thunberg, 1793). This latter, however, has a more elongated, distorted and irregular shell and, above all, is characterised by a different sexuality. Oysters are prey of several organisms, including fish, crabs, snails, starfish and flatworms but also of boring sponges, seaworms, molluscs, pea crabs and fouling in general, that can be cause irritation problems or compete for food. With regard to

disease, the protist *Bonamia ostrea* is one of the most dangerous pathogens: in 1920 it caused massive mortality events among flat oyster populations (da Silva *et al.*, 2005). These populations were then reintroduced in Europe where the disease was transferred to other established populations.

#### Reproduction

The European flat oyster is a protandric hermaphrodite (da Silva *et al.*, 2005) and shows an alternation of sexuality within one spawning season: early in the reproductive period it is male, but when it reaches the sexual maturity can alternate between the female and male stages for the rest of its life (Laing *et al.*, 2005). Males are mature after about one year of age when they release sperms into the water depending on temperature values (with a minimum of 14°C to 16°C) (Walne, 1979). Females collect sperms by using their feeding and respiration system (Laing *et al.*, 2005).

The oogenesis can produce up to 1 million eggs per spawning event, releasing them from the gonad and retaining them in the mantle cavity where they can be fertilised by externally released sperms (*i.e.*, larviparous species). After an incubation period of about 8 to 10 days, larvae develop a formed shell, a digestive system and the ciliated swimming and feeding organ (*i.e.*, the *velum*) reaching about 160 µm in size. At this point, they are released into the sea open water where they live at pelagic stage

**Table 2. Topics studied about several mollusc species and considered as promising for Italian aquaculture.**

Scientific name	Common name	Topics	Country	References
<i>Bolinus brandaris</i>	Purple dye murex	Larval rearing Semi-intensive rearing	Spain Portugal	Ramón and Flos (2001) Vasconcelos <i>et al.</i> (2012)
<i>Callista chione</i>	Smooth venus clam	Larval rearing Diet and gonadal development	Spain	Perez-Larruscain <i>et al.</i> (2011) Martínez-Pita <i>et al.</i> (2011)
<i>Donax trunculus</i>	Wedge shell	Larval rearing under different conditions Diet and lipid composition of broodstock managed in hatchery	Spain	Ruiz-Azcona <i>et al.</i> (1996) Martínez-Pita <i>et al.</i> (2012)
<i>Haliotis tuberculata</i>	Abalone	Juvenile rearing	Spain	Viera <i>et al.</i> (2011)
<i>Hexaplex trunculus</i>	Banded murex	Hatchling and juveniles	Portugal Tunisia	Vasconcelos <i>et al.</i> (2004) Labbib <i>et al.</i> (2008)
<i>Modiolus barbatus</i>	Bearded horse mussel	Genetics Artificial reproduction Rearing	Italy	Libertini <i>et al.</i> (1996) Turolla <i>et al.</i> (2002) Barile <i>et al.</i> (2011), Turolla (1998), Rossi <i>et al.</i> (1998), Pellizzato <i>et al.</i> (1998), Prioli <i>et al.</i> (1998), Turolla and Rossi (1999), Rossi (2011)
<i>Pholas dactylus</i>	Common piddock	Artificial reproduction	Italy	Rossi (2011)
<i>Venus verrucosa</i>	Warty venus	Artificial reproduction	Italy	Piccinetti (2011), Rossi (2011)



(8 to 10 days), feeding on phytoplankton for 2 to 3 weeks before settlement (Korringa, 1941, 1952; Laing *et al.*, 2005). The amount of larvae released into the seawater is correlated to the parent size, which ranges between 1.1 and 1.5 millions for oyster from 4 to 7 years old (Walne, 1979).

By contrast, *Crassostrea gigas* inverts its sex after one spawning season and releases its gametes (eggs or sperms) into the environment at one time or in small amounts over a long period (*i.e.*, oviparous species). Thus, fertilisation occurs externally and the resulting larvae develop in the seawater.

Therefore, during the larval stage life is typically planktonic and, as metamorphosis progresses, oyster moves with an extensible foot in search of a suitable substrate. Once the oyster finds it, it attaches itself by a byssus formation and then by cementation (with a physiological and morphological metamorphosis lasting 3 to 4 days) and starts its sessile life as juvenile, thus becoming spat (Laing *et al.*, 2005). From this event, the growth is quite quick for about 18 months, then it stabilises remaining constant at about 20 g of fresh weight per year and finally slows down after 5 years (Laing *et al.*, 2005). Depending on the environmental conditions, these bivalves can achieve the marketable size of 7 cm in shell length in 4 to 5 years and live in natural beds up to 20 years growing up to 20 cm of size.

#### Aquacultural activities

Oyster spat can be obtained by both wild stocks and hatchery production. Like in other bivalves, sexual maturation and subsequent reproduction is obtained by modifying the temperature of the water (*i.e.*, increasing it) and by administering the phytoplankton *ad libitum*, thus imitating the natural reproductive cycle. Compared to the conditioning of other species, the fertilisation of *O. edulis* specimens is more difficult due to a lower larval survival rate, so that a period of incubation is necessary. In general, spat is cultured using traditional techniques for bivalves at nursery stages and, when it reaches the size of 5-6 mm, it can be moved to open water to grow up.

On the contrary, natural spat harvesting is based on the employment of collectors. Some examples are mussel shells sown in density of about 30 to 60 m<sup>3</sup> ha<sup>-1</sup> (the Netherlands), or tubular nets containing mussel shells (about 600) suspended under steel frames in shallow waters (France). Recently, PVC dishes are used in intertidal areas. Therefore, seed can be transferred to the growing or fattening area. Yet, this is not always necessary for the seedling area that can also become the growing and

fattening area in some facilities. Breeding methods are generally categorised into *on-bottom* and *off-bottom*, each of them having its advantages and disadvantages. Thus, it should be better to choose the most suitable method for the selected site and for the specific financial possibility. On-bottom techniques require that oysters are seeded directly in subtidal or intertidal grounds with a stable, non-shifting bottom (Quayle, 1980), at a density of about 50-100 kg ha<sup>-1</sup>. Seeding is generally carried out in the period between May and June, when molluscs are about 1 cm long (1 year old), and here they reach the marketable size. Traditionally, cotton nets or steel frames are used to preserve the culture from predators. The on-bottom method certainly is the simplest and cheapest one, but mortality, stock loss caused by predation and siltation events are highest, and even harvesting is difficult.

On the other hand, off-bottom techniques allow oysters to be cultured in suspension. This method is certainly more expensive than the first one and requires more maintenance, but it is compensated by the rapid growth and high quality of the cultured oysters. The technique consists of using floating structures, rafts, long-line systems, suspended ropes, lanterns or plastic baskets pending from a raft/rope, where oysters are located in. Product is thinned out as it grows. Harvesting should be programmed when oysters are in their best conditions, with full and creamy meat. From on-bottom cultures, molluscs can be dredged or handily collected, whereas in the off-bottom ones they can be handily-picked. Finally, before marketed, they are temporarily stored in clean water and subjected to depuration procedures as all other bivalve molluscs.

#### Rearing in Europe

In Europe, several rearing experiments with this species have been carried out in the last decades. In particular, much attention has been paid to both survival and growth of experimental batches of hatchery-reared *O. edulis* larvae and spat (Davis and Calabrese, 1969; Laing and Millican, 1986; Spencer and Gough, 1978; Utting, 1988; Beiras and Pérez Camacho, 1994; Beiras *et al.*, 1994; Berntsson *et al.*, 1997; Rodstrom and Jonsson, 2000), and to the biochemical composition of larvae fed on different food regimes (Ferreiro *et al.*, 1990; Millican and Helm, 1994).

On the other hand, a few studies have been conducted on its reproduction (Mann, 1979; Wilson and Simons, 1985; Ieropoli *et al.*, 2005), while many growth trials were tested in different European countries like Ireland (Wilson, 1987), Germany (Pogoda *et al.*, 2011), Croatia

(Zrnčić *et al.*, 2007), Malta (Agius *et al.*, 1978), Turkey (Yildiz *et al.*, 2011), Spain (González and González, 1985; Pérez Camacho and Roman, 1985; Cano and Rocamora, 1996), and most of all Italy (Pellizzato and Da Ros, 1985; Lubrano Lavadera *et al.*, 1999; Pellizzato *et al.*, 2005; Pais *et al.*, 2007, 2012a; Carlucci *et al.*, 2010). Nevertheless, commercial farming activities of this species are still scarce in Italy. The Pacific cupped oyster *Crassostrea gigas* is preferred for aquacultural purposes although in recent years this industry has experienced high mortality rates of up to 80% due to Type 1 Ostreid Herpes Virus (OsHV-1) (EFSA, 2010).

### Grooved carpet shell

#### Distribution, habitat and exploitation

The grooved carpet shell *Venerupis decussata* (Linnaeus, 1758) is a bivalve belonging to the Veneridae family (Rafinesque, 1815). This species is found all through the Mediterranean sea, but it is widely distributed along the western Atlantic coasts from Norway to Congo and also in the northern part of the Red sea where it migrated from the Suez Canal.

This species typically lives buried in sandy and silt-muddy bottom, inhabiting the areas near and below the mean sea level (intertidal zone and subtidal zone, respectively), and buried 15 to 20 cm into the sediment. Moreover, it continuously filters surrounding water through its two siphons protruded from the substrate, picking up organic particles and phytoplanktonic cells as nourishment and to allow gas exchange between oxygen and carbon dioxide that occur with breathing. Overall, clams bear quite well the variations of chemical and physical variables of water, such as temperature, salinity, dissolved oxygen, turbidity, typical of lagoon environments or estuarine areas where they live. As a consequence, their favourite sites are generally located away from areas with high hydrodynamic, and from windy areas where the substrate where they are buried can be destabilised. Nevertheless, it is important the presence of a slight and a constant current that allows good water exchange and the constant flow of food. For this reason, clams can live on a variety of substrates although a mixture of sand, silt and granules is the most suitable composition which allows a good oxygenation and a comfortable softness of the bottom. It is important to emphasize that other filter feeders species (*e.g.* Bivalves, Hydroids, Bryozoans, Serpulids, *etc.*), can compete with a clam population for food availability. At the same time, another form of competition can take place during the recruitment, depending on the availability of suitable sub-

strates (Paesanti and Pellizzato, 2000; FAO, 2004).

In Europe, the harvesting of *Venerupis decussata* mainly occurs in countries like Spain and France, but also in Italy, especially in Sardinia, where the semi-extensive culture of the allochthonous species *Venerupis philippinarum* has been banned by the Regional Government in order to protect the native Mediterranean carpet clam (Chessa *et al.*, 2005; Pais *et al.*, 2006b).

### Reproduction

Even though occasional cases of hermaphroditism can be observed (Delgado and Pérez Camacho, 2002) especially in juvenile forms (Lucas, 1975), this clam is strictly gonochoristic and the reproduction takes place externally in the aqueous medium, mainly in summer when temperature is higher and food is abundant. Resulting larvae are freely floating for 10 to 15 days until they settle as spat (about 0.5 mm in length) and continue their growth to adult form, once they found a suitable substrate.

Like most of other marine bivalves, *Venerupis decussata* is characterised by a cyclical pattern of reproduction, which can be divided into different phases: gametogenesis and vitellogenesis, spawning and fertilisation, larval development and growth. Each bivalve species evolved a number of adaptive strategies (genetic or not) to coordinate these events with the environment in order to maximise the reproductive process (Newell *et al.*, 1982). In this regard, numerous studies show that gametogenic cycle in marine invertebrates is strictly conditioned by the interaction between exogenous factors (*i.e.*, temperature, salinity, light, availability of food, parasitic infestations) as well as by internal factors (Rodríguez-Moscoso and Arnaiz, 1998). Temperature is certainly one of the most important factors influencing the reproductive cycle (Sastri, 1975), defining both the starting point and the rate of gonadal development, whereas food availability can determine the extension of the reproductive process (Lubet, 1959). These two factors are subject to natural seasonal fluctuations and their variability is closely related to the energy available for growth and reproduction. In particular, clam reproduction requires abundant energy for providing a suitable gonadic development so that the success directly depends on ingested food or on previously stored reserves (Delgado and Pérez Camacho, 2005). In general, when food is abundant, reserves accumulated before and after gametogenesis (*i.e.*, glycogen, lipid and protein) are utilised to produce gametes when metabolic demand is high (Bayne,

1976). As a consequence, gametogenesis can differ from location to location depending on the geographic area considered: for example, in adult clams from southern Europe the cycle generally starts in March, gonads become ripe in May-June and spawning occurs in summer, following a phase of inactivity in winter (Shafee and Daoudi, 1991).

### Aquacultural activities

Until a few decades ago, the management of this species was exclusively related to the availability of natural seed. However, nowadays the manipulation of its gonadal cycle is a quite common practice. In fact, artificial spawning techniques and larval rearing programs have been recently developed. These methods are applied in highly specialised systems – the hatcheries – where breeders (previously selected from natural beds on the basis of their appearance, size and shape) are stocked into tanks for 30 to 40 days at 20°C of temperature, and richly fed with phytoplanktonic algae. In order to guarantee the continuous availability of this nourishment for breeders and future larvae, hatcheries have to possess algal culture systems.

The specimens selected are abundantly fed to maximise their gonadic maturation until they are ready to reproduction. At this phase, the release of gametes is induced by a thermal shock of the water of about 10°C (from 18 to 28°C), repeated for one or more cycles of about 30 min each. Generally, males emit before females and fertilisation occurs in small containers. The eggs obtained are counted, filtered and placed into little aquariums (about 10 L in volume), where *veliger* larvae appear after 8 days. Subsequently, they are filtered through a 100 µm mesh, daily fed with phytoplankton for the first week and then every 2 days. At the *pediveliger* stage, clams have a diameter of about 180 to 220 µm, they already have the foot but the *velum* is still present. Indeed, they spend most of their time swimming and sometimes they are fixed on the container surfaces. After about 3 weeks, the metamorphosis process is completed and the spat stage is reached (about 250 µm in size). The little molluscs can be now reared in greenhouses, fed with phytoplankton or with pumping environmental water into inland tanks, where they are placed inside small containers having a rigid mesh as bottom (*i.e.*, nursery).

From this stage onwards, methods of farming may be different depending on the features of the hatchery (*e.g.* standing water, constant water flow, downwelling and upwelling forced water flow). As said above, spat can be obtained both from natural populations in the

vernal period (digging them with sand by a small rank and riddling it to retain the seed) and from hatcheries. When a size of about 1 mm is reached, a new phase of rearing can start using a controlled system: the so-called pre-fattening. Clams grow up to about 10 to 15 mm in 2 to 4 months and it would be convenient to complete their weaning period outside, pumping natural seawater or brackish water since their maintenance into the hatchery is quite difficult for both management and for economic reasons. Once they have reached this size, depending on the preferences and the possibilities of the farmer, molluscs can be transferred to the ground (with a density of about 5000 individuals m<sup>-2</sup>) or in special facilities that allow their growth in suspension, such as net bags (*pôches*) or stacked baskets (at lower density). Moreover, if they are sown directly on the substrate, it is advisable to protect the seed from predation by plastic nets. In this way, clams are able to attain a size of 20 to 25 mm in about 2 months. At this phase, management regards only the preparation and maintenance of breeding substrate (*i.e.*, cleaning and removal of algae or predators) or the control of the suspension systems (*i.e.*, attachment and clearing of encrusting organisms or fouling).

The last procedure of the production cycle is fattening, *i.e.*, when carpet shells grow in the bottom within the sediment. In this way, molluscs live following their natural pattern, filtering water and then feeding until they achieve the commercial size of at least 25 mm in length. According to the environmental conditions and breeding, the fattening stage can be completed in a period of 12 to 28 months. After reaching the commercial size, clams can be gathered in different ways depending on the type of farming. When and where it is possible, fishermen manually collect the bivalves by walking using a rake equipped with an appropriate net, whose mesh is sized to hold the molluscs and allow the escape of the sediment. Alternatively, the harvest can be made from boats (with oars or engines) furnished with an extended rake.

### Rearing in Europe

During the last decades, clam aquaculture has conspicuously developed in Europe and particularly in Italy where, after its introduction into the northern Adriatic lagoons, the Pacific carpet clam *Venerupis philippinarum* (Adams and Reeve, 1850) has been intensively exploited due to its rapid growth and propagation (Paesanti and Pellizzato, 2000). On the contrary, farming activities of the grooved carpet shell *Venerupis decussata* are still limited at

present, although intensive research on the rearing of this species has been carried out throughout the continent. In particular, several studies have been conducted both on gametogenesis (Xie and Burnell, 1994; Rodríguez-Moscote and Arnaiz, 1998; Ojea et al., 2004; Serdar and Lök, 2009) and reproductive cycle (Breber, 1980; Beninger and Lucas, 1984; Laruelle et al., 1994; Urrutia et al., 1999; Delgado and Pérez Camacho, 2003, 2007) of this species.

Much research has been carried out also on broodstock conditioning (Ojea et al., 2008; Matias et al., 2009) and on diets for *Venerupis decussata* larvae (Pérez Camacho et al., 1994; Matias et al., 2011) and seed (Albentosa et al., 1996a, 1996b, 1996c, 1997, 1999; Lamela et al., 1996; Jara-Jara et al., 1997; Fernández-Reiriz et al., 1998, 1999; Pérez Camacho et al., 1998, 2007; Enes and Borges, 2003) in controlled conditions. Furthermore, a number of successful trials have demonstrated the feasibility of rearing this bivalve in different European countries (Walne, 1976; Breber, 1985; Pastore et al., 1996) using different culture techniques in the sea as well as in coastal lagoons (Chessa et al., 1998, 2005; Pais et al., 2006b; Serdar et al., 2007).

## Razor clams

Only two genera of razor clams are commercially exploited in Europe: genus *Ensis* (*E. arcuatus*, *E. minor*, and *E. siliqua*), belonging to the Pharidae family; and genus *Solen* (*S. marginatus*), belonging to the Solenidae family. The razor clams have a high and increasing commercial value due to the high prices reached in European and international markets (Barón et al., 2004). Spain, Italy, France, Portugal and the Netherlands are considered as the most important countries involved in this market. In 2004, the import value of the razor clam market within the EU25 was 550 million € (BIM, 2005). The world landings of these species are low compared with other traditional shellfish species (i.e., oysters, scallops, or clams), and the fishing pressure on wild populations is increasing. Signs of severe exploitation of natural stocks are documented (Gaspar et al., 1998; Tuck et al., 2000; Fahy and Gaffney, 2001) also in Italy, where razor clams (*E. minor* and *S. marginatus*, a less valuable species) are widely distributed, and the quantity harvested from the wild is decreasing. This species is of interest for aquaculture both for improving natural stocks and for producing food. The aquaculture potential of *E. arcuatus* and *S. marginatus* has been recently assessed by a number of specific trials carried out mainly in Spain, a country importing large quantity

of this seafood (47% of the European importation value, according to data obtained from Eurostat information database). The experiments carried out on the production of razor clam species diffused in Spain, using hatchery and semi-intensive aquaculture techniques, are deeply summarised in a recently published report (Guerra Diaz et al., 2011).

Despite interest in razor clam aquaculture, little is known about its growth and reproduction, even though the studies of the cultivation of 3 razor clams (*S. marginatus*, *E. siliqua* and *E. arcuatus*) date back to 1990. Available literature is scarce and mainly represented by documents for internal use (reports, MSc, or PhD thesis), or by posters and short presentations documented as short abstracts in international or, more often, national meetings. The availability of these last documents is reduced also for the use of native languages other than English. Some documents are in form of grey literature, thus reducing the possibility for exchanging results among researchers. Currently, no information is available about the aquaculture potential of *E. minor*, which is located exclusively in the Mediterranean sea basin.

## Reproduction, larval and post-larval rearing

In *Solen marginatus* the spawning takes place in a few weeks during spring, in *Ensis minor* in March-April in the southern Adriatic sea, while in *E. siliqua* there is only one spawning period (May-June) (Guerra Diaz et al., 2011). The increase of seawater temperature during broodstock conditioning helps the maturation process in most of the species, except for *E. arcuatus* that is conditioned to ripeness at low temperature. In some species (*S. marginatus*, *E. siliqua*), the ripe adult can be successfully induced to spawn using thermal shock (Loosanoff and Davies, 1963; Martínez-Patiño et al., 2007; da Costa and Martínez-Patiño, 2009), while in *E. arcuatus* the change of water levels by simulating tides is the only effective method (da Costa et al., 2008).

The management of eggs for fecundation and of larvae during rearing can be carried out according to the same protocol utilised for other bivalve species. Larval culture duration is very short in *S. marginatus* (8 days), due to the high levels of stored reserves in eggs (da Costa et al., 2011b), and a larval survival ranging from 28 to 81% (53% on average) was recently achieved by da Costa and Martínez-Patiño (2009) in specimens obtained from adults spawned in hatchery by induction. As a consequence of the large size of eggs and the short length of the larval stage, a different pat-

tern in the use of gross biochemical and fatty acid reserves during larval development compared to other razor clams and bivalve species was recently found (da Costa et al., 2011b). A dramatic reduction in survival was obtained by the same authors in 1-month-old spats (8.6%), the bottleneck phase resulting at the post-larvae age corresponding to 1 mm in length (15 to 22 day from settlement). Using seed 1.3 mm in length, da Costa and Martínez-Patiño (2009) achieved better survival when the rearing was done without substrate than when 2 types of sand were utilised. In *E. arcuatus*, recently da Costa et al. (2011a) achieved the settlement of larvae on day 20, a survival from egg to newly settled postlarvae ranging between 4.8 and 24.8% (14.35% on average), and a very low (4.8%) survival from settlement (day 20) to 3-month-old spat (15.5 mm in length).

## Grow-out

The effect of substrate (fine-grain sand or coarse sand, 150 to 600 or 300 to 1200 µm grain diameter, respectively) in comparison to the absence of substrate was tested by da Costa et al. (2011a) in seed culture of *E. arcuatus* (3.76 mm in length) in nursery during a experiment lasting 30 days. No differences among treatments resulted for length, while the fine sand induced a lower weight and the absence of substrate a lower survival. Substrate and stocking density influenced the *E. arcuatus* juveniles performance (length and survival), that resulted the worst at high density (36 g per a 5 L bottle) and with fine sand as substratum (*vs* coarse sand). The on-growing in cage buried in sand, in natural environment, from the initial size of 60 to 80 mm highlighted high mortality and high sensitivity to changes in salinity. For those reasons, the choice of the site is highly affecting juveniles grow-out and performance.

Da Costa and Martínez-Patiño (2009) in *S. marginatus* broodstock from the wild managed spawning induction and fecundation, larval and spat rearing in hatchery, and assessed growth performance of juveniles produced in hatchery when transferred to natural beds. Two-year-old razor clams showed a survival ranging from 50 to 83% and after 2 to 3 years from seeding they reached the commercial size (80 mm). Since some of those specimens were utilised as broodstock, the culture cycle of *S. marginatus* was closed. In this species, a greater tolerance for salinity variations was found in comparison to *E. arcuatus*.

In experimental condition, *E. arcuatus* reached a length ranging from 90 to 100 mm (da Costa et al., 2011a) after two years of cultivation, while *S. marginatus* reached the com-



mercial size only after 3 years (da Costa and Martinez-Patiño, 2009). In *E. minor*, Froggia (1975) found that the size of 100 mm was reached in 2-year-old individuals. After 2 years, the growth is reduced as a consequence of the gametogenic activity both in *E. arcuatus* and in *S. marginatus*.

#### Critical points

Among the limiting factors for aquaculture of razor clams, the followings can be listed: short reproduction period, requirement of substrate and, for the on-growing phase, appropriate seed holding systems, low survival times in absence of soft substrate, difficulties for checking growth due to burying behaviour, need for appropriate substrate. At the moment, postlarval and seed survival obtained is very low for *S. marginatus*, and mainly for *E. arcuatus*. For *S. marginatus*, low survival during the on-growing of juveniles has been also achieved. The information related to larval and postlarval nutrition and their influence on growth and survival presently is very scarce. In addition to these aspects which constitute a major bottleneck for the reproduction management, the requirement of an appropriate substrate for the culture process can also be envisaged as an obstacle for promoting the development of culture in hatchery. Therefore, further investigations on the specific requirements at different stages of the development for the diverse species could improve the results and the potential for culture of razor clams.

### Octopus

#### Statistics

The benthic octopuses of the family Octopodidae (Order: Octopodida, Leach, 1818) are one of the most familiar groups amongst the cephalopods. In this family, there are over 200 species, which range in size from pygmy *taxa* mature at <1 g (e.g., *Octopus wolfi*) to giant forms exceeding 100 kg (e.g., *Enteroctopus dofleini*). These species inhabit all marine habitats from tropical intertidal reefs to polar latitudes and in the deep sea to nearly 4000 m (Villanueva and Norman, 2008). Only a few cephalopods are commercially fished on a large scale (Kreuzer, 1984): squid (genus *Loligo*) is by far the main species, representing 73% of cephalopod world catches; cuttlefish (genus *Sepia*) is the second with 15%; and octopus the third with 8.8%. *Octopus vulgaris* Cuvier, 1797 (order Octopoda, suborder Incirrata) is one of the most important species in terms of commercial value and landings. Thirty-one per cent of the total production is put out by Mexico (11,855 t), which is

the world leader, followed by Portugal (9,965 t; 27%), Spain (5,792 t; 16%) and Italy (3,018 t; 8%) (FAO, 2010).

#### Potential for aquaculture

*Octopus vulgaris* presents many characteristics: easy adaptation to captivity due to their benthic mode of life, reclusive behaviour, and low swimming activity; high growth rates (Aguado-Giménez and García García, 2002; Iglesias *et al.*, 2006, 2007) (between 3 and 15% body weight day<sup>-1</sup>); high food conversion rates (incorporating 40 to 60% of ingested food into tissue) (Mangold and Boletzky, 1973); high fecundity (producing from 100 to 500 thousand eggs per female) with well developed hatchlings compared to other molluscs; high market size and price (García García *et al.*, 2004) in areas where cephalopod consumption is high; and a non-geographically limited request by the markets (Berger, 2010).

The life cycle of this octopus species was carried out for the first time in captivity by Iglesias *et al.* (2002) using *Artemia* and zoeae of spider crab (*Maja squinado*) as live feed. The authors obtained a survival of 31.5% day<sup>-1</sup> post-hatching, a weight of 0.5 to 0.6 kg in 6-month-old animals and of 1.6 kg in 8-month-old animals (Iglesias *et al.*, 2002).

#### Reproduction

Octopuses are dioecious. *Octopus vulgaris*, as other benthic octopuses (*O. mimus*), produces numerous small eggs (2.7 mm in length) that hatch into planktonic, free-swimming hatchlings (1.2 mg) very different in their morphology, physiology, ecology and behaviour compared to the adult stage. Other octopuses (i.e., *O. maya*, *O. bimaculoides*), by contrast, produce relatively few, large eggs resulting in better developed hatchlings with a benthic habit that resembles the adult (Villanueva and Norman, 2008). They readily mate in captivity and easily spawn, and females reared to the sexual maturity can produce viable spawns (Iglesias *et al.*, 2000, 2004). No further information is available about reproduction in captivity. However, reproduction is not considered as a limiting factor for this species and Iglesias *et al.* (2000) recommend a correct feeding of broodstock before mating and a correct management of females after eggs deposition. The optimal water conditions for broodstock (maintained at a 1:1 ratio of males and females or 1:3 ratio, according to Iglesias *et al.*, 2007) are temperature ranging from 13°C to 20°C and salinity around 32-35 PSU. Normally rectangular tanks (5 to 10 m<sup>3</sup>), maintained with low light to obtain spawning as swiftly as possible, have

been utilised (Iglesias *et al.*, 2000; De Wolf *et al.*, 2011) and a diet based on frozen crustaceans (80%), fish (15%) and bivalve molluscs (5%) seemed to be favourable to obtain highly viable spawns (Iglesias *et al.*, 2002). Males show a copulatory activity and egg deposition occurs on the walls and on the roof of den where the broodstock move. The eggs, cared for by the females, hatch after 34 days at 20±1°C, in the Mediterranean area (Villanueva, 1995), while more time is taken for hatching in captivity as the results obtained by Iglesias *et al.* (2000) showed.

Currently, the rearing of paralarvae is not possible, and the impossibility of obtaining benthic juveniles on a commercial scale in captivity (Navarro and Villanueva, 2000, 2003; Iglesias *et al.*, 2004, 2007) forces farmers to catch from the wild the juveniles that will be utilised in industrial on-growing. This behaviour is highly objectionable from an ethical point of view because it potentially increases fishing pressure on octopus stocks.

#### Larval rearing and feeding

Many rearing systems have been experimented, different for tank colour, size and shape, larval and prey densities and environmental factors (light, water flow and temperature), resulting in different survival of paralarvae. However, the phase of paralarvae rearing is considered inadequately explored and survival of paralarvae older than 50 days post-hatch in captivity conditions is very rare, and referred only by Iglesias *et al.* (2002, 2004) that reared octopus until 8 months by *Artemia* and zoeae. In Italy, De Wolf *et al.* (2011) carried out many trials from 2003 to 2007. They produced eggs and paralarvae in captivity and reared juveniles that reached the age of 160 days, by using standard aquaculture procedures for feeding (based on *Artemia* and rotifers as live prey) and rearing paralarvae. Currently, the most recognised opinion is that nutritional aspects are the most important factor influencing the performance and mortality of paralarvae (Iglesias *et al.*, 2007). For feeding paralarvae different prey were experimented: *Palaemon serrifer* zoeae (Itami *et al.*, 1963), *Artemia* (Imamura, 1990), *Artemia* enriched with *Nannochloropsis* sp. (Hamazaki *et al.*, 1991) or *Tetraselmis suecica* or *Chlorella* sp., *Liocarcinus depurator* and *Pagurus prideaux* (Villanueva, 1994, 1995; Villanueva *et al.*, 1995, 1996), *Artemia* with spider crab *Maja brachydactyla* zoeae (Moxica *et al.*, 2002; Iglesias *et al.*, 2004), *Artemia* supplemented with copepods or juvenile mysids. Rotifer *Brachionus plicatilis* too was recently tested (De Wolf *et al.*, 2011). The survival rate largely varied with



prey (species, strain, size, culture technique) and with trial, usually resulting very low after 1 or 2 months from hatching: 8.3% (Moxica *et al.*, 2002) and 0.8 to 9% (Villanueva, 1994, 1995; Villanueva *et al.*, 1995, 1996), respectively. As regards to live prey, spider crab was preferred and induced better performance, probably for its high content in the essential fatty acids (EFA), particularly in arachidonic acid (ARA) (Iglesias *et al.*, 2007). A mix of *Artemia* and inert diet (Navarro and Villanueva, 2000), and millicapsules (Villanueva *et al.*, 2002; Navarro and Villanueva, 2003) was also tested, thus obtaining reduced growth performance and a survival limited to first month.

Even though much research was carried out on this topic, high mortality and scarce growth performance have been obtained. At the moment, the high diversity of the experimental protocol of the trials and the lack of standardised methodologies do not let find the most effective diet for adequately feeding paralarvae. The low performance of this stage of culture can be also due to inappropriate techniques utilised during rearing. An inadequate size of tanks and the hydrodynamic behaviour of water can be responsible for mortality or damages in paralarvae arms and mantle (Rasmussen and McLean, 2004). In large volume tanks, higher survival and longevity was obtained by De Wolf *et al.* (2011) and better growth by Sanchez *et al.* (2011), the larger volumes mitigating the fluctuations in water temperature and in other water parameters. A drastic reduction of survival (4 to 0.6%) was noticed when density increased from 3 to 15 ind. L<sup>-1</sup> (De Wolf *et al.*, 2011). The negative effect of high density could be explained by the releasing of paralysing substances or by the competition for space during prey location and capture.

Useful information was obtained about the nutritional requirements of paralarvae by the comparison of the chemical profiles of mature ovaries, eggs at different developmental stages, fresh hatchlings and wild juveniles with the chemical profiles of paralarvae cultured for one month with different live *natural* prey and with those of the prey administered (Navarro and Villanueva, 2000, 2003; Villanueva *et al.*, 2002, 2004, 2009; Villanueva and Bustamante, 2006). Special attention was paid to polyunsaturated fatty acids (PUFA), especially docosahexaenoic (DHA), DHA/eicosapentaenoic (EPA) ratio, phospholipids, cholesterol, to some essential amino acids (lysine, leucine and arginine representing about 50% of the total essential amino acids of paralarvae), mineral (copper) and vitamins (Vitamin A, but especially Vitamin E; Villanueva *et al.*, 2009). The requirement of

Vitamin E, which is higher than for other marine molluscs and fish larvae, is probably due to the high percentage of PUFA in paralarval and juvenile cephalopods, that consequently need of a strong antioxidant system.

### Grow-out

The on-growing of octopus started in Galicia in the mid-1990s with wild sub-adults 750 g in weight. The first findings on on-growing experimental trials date at the end of the last century and have been obtained in Spain (Iglesias *et al.*, 1997, 2000), Portugal, and Italy (Cagnetta and Sublimi, 2000).

### Rearing parameters

Much information on the behaviour of octopus, which can be usefully used for an appropriate design of tanks and equipment for rearing, is obtained by observing octopus in laboratory or directly in nature.

Even though octopus shows a rapid and easy adaptation to life in captivity (Iglesias *et al.*, 2000) in different kinds of containers [aquaria, cylindrical-conical containers, raceways, floating cages and also benthic cages, as recently showed by Estefanell *et al.* (2012)], the habit to attach to any surface can be a problem for handling the specimen maintained in captivity, also as a consequence of their tendency to escape from containers. To counteract the aggressive behaviour and to respect the natural habits of octopuses that prefer dark sites, inside tanks or floating cages, shelters should be put in adequate number. The feeding habits and the utilisation in captivity of live food or dead whole marine organisms need frequently cleaned tanks or the utilisation of self-cleaning tanks (Vaz-Pires *et al.*, 2004).

About water quality parameters, octopus shows a very low tolerance to low concentrations of salts, normally living in a range fluctuating from 35 to around 27 g L<sup>-1</sup> (Boletzky and Hanlon, 1983). For on-growing, the temperature should be kept between 10°C and 20°C, and better performances are achieved at the higher temperatures in this range. Temperature above 23°C can be responsible for high mortality, so that rearing in the Mediterranean area can be critical in summer months. This parameter affects the most important zootechnical parameters (growth, food conversion and ingestion).

The results obtained by Cerezo Valverde *et al.* (2003) highlighted that ammonia excretion is very important in this species compared to finfish, such as sea bass or sea bream. Thus, checking this parameter during rearing is fundamental. The same can be said for oxygen

(Cerezo Valverde and García García, 2004), mainly in *post-prandial* period (from 6 to 16 hours after the meal ingestion), due to high consumption found in octopus. At a temperature of 17°C to 20°C, the optimum oxygen saturation resulted ranging from 100 to 65%, sub-optimal saturation from 65 to 35%, dangerous below 35%, and lethal below 11% of saturation (Cerezo Valverde and García García, 2005). *Octopus vulgaris* shows a great resistance to hypoxia, similarly to other cephalopod species. The resistance to low oxygen levels was higher in small sized octopuses and at low temperature, this parameter playing an important role by increasing the lethal oxygen value. Given the relevant changes produced in the most suitable oxygen levels, temperature variations are highly critical for octopus. For this reason the utilisation of benthic cages could reduce the abrupt changes of environmental parameters associated with rearing in floating cages (Estefanell *et al.*, 2012). Males and females showed a different behaviour, the latter having greater oxygen consumption, conversely to what the same authors previously found on immature males and females (Cerezo Valverde and García García, 2004).

The separation according to sex improved culture yield, as the non-fertilised females continued to grow to commercial size without interferences due to the stopping of feeding during eggs care. The rearing performance of female can be further improved by using high light intensity in order to delay the sexual maturation and to achieve a greater somatic growth (Iglesias *et al.*, 2002), because egg formation generates higher metabolic requirements during sexual maturation of females (O'Dor and Wells, 1978). In other experiments, differences in growth and food intake between males and female were not found (Aguado-Giménez and García García, 2002).

In relation to the hierarchical behaviour of octopus, the influence of rearing density has been studied. Domingues *et al.* (2010) tested growth and survival during on-growing of octopus at 3 densities (4 vs 8 vs 15 kg m<sup>-3</sup>) in an experiment lasting 70 days. No differences were found for growth, but a lower survival characterised the highest density group. Even though the groups showed similar feeding rates, the food conversion rates were lower in medium and high density octopuses, probably for their more stressing and uncomfortable condition. Previously, Otero *et al.* (1999) tested 10 and 20 kg m<sup>-3</sup> as stocking density, suggesting a density no higher than 10 kg m<sup>-3</sup> to obtain the best growth performance. A modulation of density in relation to water temperature (higher density at lower temperature) and to

culture system (higher in cage than in tank) should be considered.

### Feeding

Lee (1994) studied the feeding behaviour of cephalopods and highlighted the importance of visual stimuli, chemical and textural properties of food, and nutritional quality of the diet, this last affecting the ingestion. Octopuses prefer live food, giving better performance with mixed diet than with monodiet. Cagnetta and Sublimi (2000) obtained a better growth when crabs were used in comparison to a diet based on squid or fish, even though squid simplify the management of tank for cleaning, due to reduced waste. In a recent paper, Estefanell *et al.* (2012) showed how feeding octopuses on monodiet based on bogue (*Boops boops*) discarded from fish farms produced high growth (1.8 to 1.9% day<sup>-1</sup>) and high survival (91 to 97%), and the best biomass increment (178 to 212%) and food conversion rates (2.3 to 2.6) ever recorded for *O. vulgaris* under industrial rearing conditions. The species can be easily adapted to dead food (Boucaud-Camou and Boucher-Rodoni, 1983) and to commercial dehydrated foods, as pelleted diets (Lee, 1994), that can be appreciated and ingested in relation to the specific texture characteristics. Different digestibility for lipids (46%) and proteins (96%) justifies the nutritional superiority of food poorer in lipids and richer in protein that is the main source of energy in octopus (Lee, 1994; O'Dor *et al.*, 1983). The knowledge of amino acid composition of food could be essential for evaluating the adequacy of protein to satisfy energetic requirements. Crabs are also preferred to fish for specific pre-ingestional (size, shape, texture, flavour) and/or post-ingestional stimuli (digestibility, assimilation or energetic benefit) that this food can generate (Lee, 1994). The high variability of natural food tested in trials referred in different papers is responsible for the large differences found in documented growth responses and for non-consistent results (Aguado-Giménez and García García, 2002).

Even if research on artificial diets for cephalopods started 2 decades ago (in the early 1990s), the lack of an appropriate artificial diet for nutritional requirements of octopus still represents a limiting factor for its fattening phase.

### Quality

The shelf life of octopus is very short, even at low positive temperatures, due to high protein deterioration which is responsible for an increase in nitrogen released during storage. Hurtado *et al.* (1999) reported a shelf life lasting 6 to 7 days after catch at 2.5°C and Barbosa

and Vaz-Pires (2003) found a shelf life of 10 days at 0°C. Different methods and different parameters have been tested for monitoring quality and quality evolution during the shelf life, based on physical, chemical, and microbiological analysis. A sensorial scheme based on the Quality Index Method was developed by Barbosa and Vaz-Pires (2003). In their trial, the authors found that the rejection of octopus kept in crushed ice occurs at day 8. Several attempts were carried out to increase the shelf life by an appropriate treatment (high pressure, heat combined to high pressure, gamma radiation) and to improve the textural properties inducing the softening of meat (Hurtado *et al.*, 2001a, 2001b, 2001c; Sinanoglou *et al.*, 2007). Recently, Mendes *et al.* (2011) tested the active packaging based on soluble CO<sub>2</sub> stabilisation (SGS) methodology to obtain ready-to-eat products. Even though no extension of shelf life was obtained, the bacteriostatic effect allowed an effective extension of the period of use by date. Besides the short shelf life, octopus shows other peculiarity as regards its quality, for example the high solubility of fibrillar protein, responsible for a considerable leaching when processing in water is performed, reducing nutritive value and affecting sensorial characteristics.

The edible portion of octopus is very high, compared to fish or crustaceans (80 to 85 vs 40 to 75 vs 40% to 45%) (Kreuzer, 1984). Also, its composition presents very low content in fat (0.54 to 0.94%, according to the season), where the total polyunsaturated fatty acids of the n-3 species (PUFA<sub>n-3</sub>) range between 42 and 47% (Ozogul *et al.*, 2008), and EPA and DHA represent a high percentage of total fatty acids (Zlatanov *et al.*, 2006; Ozogul *et al.*, 2008).

### Critical points

The high market request in the Mediterranean, Latin America and Asian countries, the declining landings by fishery, the features of subadults and adults constitute excellent starting points for proposing octopus as a new candidate for aquaculture. Currently, the production from aquaculture is constantly increasing, even though it is still scarce (30 t).

Some reviews summarising useful information for the different steps of the octopus cycle in aquaculture conditions are available. Unfortunately, the results achieved until now are not conclusive and some bottlenecks reduce the feasibility of farming this species in a manner completely independent from the wild.

The high mortality in the paralarvae stage (Iglesias *et al.*, 2006) and the inexistence of adequate artificial feeds for paralarvae and subadults (Domingues *et al.*, 2005, 2006, 2007;

Rosas *et al.*, 2007; García *et al.*, 2011) currently are the two major bottlenecks for the commercial aquaculture of this species (García García and García García, 2011). Evaluating the economic viability of *O. vulgaris* on-growing, García García *et al.* (2004) found that juveniles represented around 41% of total costs. Even though that incidence is highly variable depending on catches, the on-growing activity is now retained a high risk business with low profits. The success of paralarval rearing in order to achieve economic and environmental sustainability for octopus aquaculture is essential.

The management of this species in captivity (*i.e.*, during the different steps of the culture until slaughtering) should take into account that, like other cephalopods, *O. vulgaris* has some behavioural characteristics (Mather, 2008) which highlight the needs for the animal welfare consideration. In UK, Canada and Australia an appropriate legislation is already at stake (Berger, 2010).

## Purple sea urchin

### Distribution, habitat and exploitation

The purple sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) is widely distributed in the Mediterranean sea and along the North-eastern Atlantic coast, from Scotland and Ireland to southern Morocco (Boudouresque and Verlaque, 2001). This sea urchin lives on rocky substrates and in seagrass meadows, from shallow waters down to about 20 m depth. It is a species of commercial importance, with a high market demand for its roe, particularly in the Mediterranean basin (Régis *et al.*, 1986) and more recently in other European non-Mediterranean areas (Byrne, 1990; Barnes and Crook, 2001). In the last decades, its populations have shown a wide scale decline in many European countries due to overfishing (Boudouresque and Verlaque, 2001). In Italy, the harvesting of *P. lividus* is a widespread activity mainly exerted in southern regions (Tortonese, 1965; Guidetti *et al.*, 2004; Gianguzza *et al.*, 2006; Pais *et al.*, 2007) and, despite the fishery of this species being regulated by a number of decrees (*i.e.*, fishing periods, minimum size of harvestable individuals and quantity per day per fisherman), the harvesting of *P. lividus* is intensively practised, particularly due to high tourism demand. For this reason, shallow rocky reef populations of *P. lividus* are mainly exploited by both authorised fishermen and poachers equipped with SCUBA, but also by occasional collectors throughout the year (Pais *et al.*, 2012b).

## Reproduction

In general, *Paracentrotus lividus* biology has been well studied, and much research has been carried out to determine all the phases of the reproductive cycle from many European countries (Byrne, 1990; Lozano *et al.*, 1995; Spirlet *et al.*, 1998b; Sanchez-Espana *et al.*, 2004; Pais *et al.*, 2006a). This Echinoid has external fertilisation and gametes are released in the water. The development of the embryo is quite fast and is a multi-phase process.

In controlled conditions, adult sea urchins are taken out from the rearing seawater (18°C to 20°C) in order to obtain gametes. To do so, 0.5 to 1 mL (according to the specimen size) of a  $5 \times 10^{-1}$  M KCl solution are injected inside the mouth using a syringe. Afterwards, sea urchins are gently rotated putting them upside down on glass beakers containing sterilised and ultra-filtered (Millipore filters 0.22 µm) (Millipore Co., Billerica, MA, USA) seawater and the gonadal products (orange-red eggs from females and white emulsion from males) are emitted from the genital pores. After cleavage, the larval period can last differently, and metamorphosis may be delayed due to environmental features. In fact, the ciliary movement of the larvae can be negatively affected by several factors and, consequently, may influence their feeding ability. Generally, however, the *echinopluteus* stage is reached within 48 hours and if the *echinoplutei* are properly fed, they can undergo the metamorphosis within 3 weeks (Yokota *et al.*, 2002). Subsequently, the newly formed juveniles can settle in the substrate and their benthic phase begins.

## Aquacultural activities

In case of severe depletion of *Paracentrotus lividus* wild stocks, aquacultural practices could represent a valid alternative to fishing (Fernandez and Pergent, 1998). Actually, as several recent studies demonstrated the possibility of improving cultivated edible sea urchin gonadal quality (Spirlet *et al.*, 2000; Shpigel *et al.*, 2005), local and tourism roe market demand could be supplied by industrial applications of similar techniques. Furthermore, the optimisation of *P. lividus* gametogenesis (Shpigel *et al.*, 2004; Luis *et al.*, 2005), if aimed at obtaining fertilised eggs, larvae and juveniles, could be useful to test restocking practices in the most severely exploited areas.

As described above, *P. lividus* artificial reproduction is a very easy practice. In contrast, the subsequent rearing phases are to some extent more difficult to carry out. Post-metamorphic juveniles can be fed using different algal species (Cellario and Fenaux, 1990; Grosjean *et al.*, 1996, 1998) until their mean

individual size reaches 3 to 5 mm. However, since the growth of the juveniles is not homogeneous, sea urchins are graded regularly, and those with a diameter larger than 5 mm are transferred into *pre-growing* nurseries. Subsequently, the subadults (*i.e.*, specimens whose size exceeds 10 mm, but it is below the minimum marketable size of 40 to 50 mm) are positioned in rearing baskets with all sides made out of mesh. These rearing baskets are suspended in tanks to allow a good seawater circulation around and inside them, and an effective removal of solid wastes produced by the sea urchins. In general, during the growing phase sea urchins are fed *ad libitum* with fresh algae (or, in alternative, with artificial diets) twice a week. The cleaning of the baskets and tanks is regularly done and dead specimens are daily removed. Furthermore, sorting of sea urchins is done to divide the batches into different size classes. During the entire production cycle, a photoperiod of 12h/12h light/dark is usually used.

## Rearing in Europe

In the past decades, several studies carried out in Europe have pointed out the possibility of successfully rearing this Echinoid. Indeed, much research has been done to reproduce the entire life cycle of *Paracentrotus lividus*, since aquaculture techniques have the potential for production of this species for both human consumption and for its use as model in research in developmental biology. Therefore, a number of studies have been conducted on the feeding preferences of juvenile and adult sea urchins and, as these echinoderms are herbivorous, they have focused on the use of several algal species (Le Gall, 1989; Frantzis and Grémare, 1992; Boudouresque *et al.*, 1996; Lemée *et al.*, 1996; Grosjean *et al.*, 1996, 1998; Cook and Kelly, 2007a; Cook *et al.*, 2007). On the other hand, the use of different artificial diets has been tested in trials aimed to improve gonadal quality and somatic growth of the sea urchins (Fernandez, 1997; Basuyaux and Blin, 1998; Fernandez and Pergent, 1998; Spirlet *et al.*, 1998b, 2001; Fernandez and Boudouresque, 2000; Pantazis, 2009; Fabbrocini *et al.*, 2011). In addition, a number of polyculture systems have been tried out in small-scale rearing experiments in order to enhance the production performances of *P. lividus* (Schuenhoff *et al.*, 2003; Cook and Kelly, 2007b, 2009).

At present, however, despite all the above mentioned farming experiences, no rearing activities of commercial importance are present in Europe. The only exception is represented by the Dunmanus Seafood Ltd. (Durrus, Ireland), in which hatchery-reared juveniles of this

species are grown to market size mainly by ranching and are then seeded to rock pools or subtidal areas (Kelly and Chamberlain, 2010).

## Conclusions

Shellfish culture can be regarded as a positive example of aquaculture activity. In particular, bivalve culture can be looked at as a paradigmatic example of sustainable economy safeguarding the environment. Indeed, it is at a low trophic level, it does not require the provision of feed input, it can help to reduce the level of water eutrophication, and it is suitable for integrated forms of aquaculture [*i.e.*, integrated multi-trophic aquaculture (IMTA)].

Nevertheless, we cannot hide or leave out some criticism on this activity, such as the environmental consequences due to the translocation of non native species which in turn are vectors of coastwise introduction of non-indigenous species (protists, algae, macroalgae, invertebrates, *etc.*), the impact generated on benthic communities (when the intensiveness of the culture is very high), or due the tools utilised for harvesting (as in the case of burrowing species).

The two sides of the same coin dictate more careful choices than those made in the past. These new choices should favour the cultivation of native species and avoiding the translocation of seed, juveniles and adults. This approach can be an economic driver, stimulating the development of a complete production chain, enhancing the creation of hatchery for spat production and reducing the potential dependence from other countries for seed supply. Although significant improvements could be achieved through specific actions on the species traditionally cultured, the way forward for the future is the culture of new species. The above survey, focused on the perspectives offered by some mollusc native species and the echinoderm *Paracentrotus lividus*, shows that we can make a virtue of necessity. This can be done thanks to research and experimentations carried out primarily in other countries that insist on the Mediterranean, but also in Italy where the transition from the experimental trials to the effective culture could be immediate for some species (*i.e.*, flat oyster and grooved carpet shell), or less immediate but certainly feasible for other species (*i.e.*, razor clams, octopus and purple sea urchin) for which we are still in a more or less advanced development of the whole culture process.

Therefore, if we move from theory to practice, enormous economic and environmental



benefits can be achieved through the creation of new activities, the demand for new jobs, an expanded product offer for the market and, simultaneously, a reduced impact on natural resources that the traditional fishery for shellfish species of commercial importance notoriously produces.

## References

- Agius, C., Jaccarini, V., Ritz, D.A., 1978. Growth trials of *Crassostrea gigas* and *Ostrea edulis* in inshore waters off Malta (Central Mediterranean). *Aquaculture* 15:195-218.
- Aguado-Giménez, F., García García, B., 2002. Growth and food intake models in *Octopus vulgaris*, Cuvier (1797): influence of body weight, temperature, sex and diet. *Aquacult. Int.* 10:361-377.
- Albentosa, M., Fernández-Reiriz, M.J., Pérez-Camacho, A., Labarta, U., 1999. Growth performance and biochemical composition of *Ruditapes decussatus* (L.) spat fed on microalgal and wheatgerm flour diets. *J. Exp. Mar. Biol. Ecol.* 232:23-37.
- Albentosa, M., Labarta, U., Fernández-Reiriz, M.J., Pérez-Camacho, A., 1996a. Fatty acid composition of *Ruditapes decussatus* spat fed on different microalgae diets. *Comp. Biochem. Phys. A* 113:113-119.
- Albentosa, M., Pérez Camacho, A., Beiras, R., 1996b. The effect of food concentration on the scope for growth and growth performance of *Ruditapes decussatus* (L.) seed reared in an open-flow system. *Aquacult. Nutr.* 2:213-220.
- Albentosa, M., Pérez-Camacho, A., Labarta, U., Fernández-Reiriz, M.J., 1996c. Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters. *Aquaculture* 148:11-23.
- Albentosa, M., Pérez-Camacho, A., Labarta, U., Fernández-Reiriz, M.J., 1997. Evaluation of freeze-dried microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters. *Aquaculture* 154:305-321.
- Alcaraz, M., Dominguez, M., 1985. Larvas de moluscos lamelibranquios en la ría de Pontevedra (N. de España): ciclo annual. *Invest. Pesq.* 49:165-173.
- Barbosa, A., Vaz-Pires, P., 2003. Quality index method (QIM): development of a sensorial scheme for common octopus (*Octopus vulgaris*). *Food Control* 15:161-168.
- Barile, N.B., Turolla, E., Antonetti, L., Scopa, M., Cappabianca, S., Mascilongo, G., Nerone, E., Recchi, S., 2011. Pilot project for the production of seed from commercially valuable molluscs: *Modiolus barbatus* (Bearded mussel). *Ovidius Univ. Ann. Chem.* 22:47-56.
- Barnes, D.K.A., Crook, A.C., 2001. Implications of temporal and spatial variability in *Paracentrotus lividus* populations to the associated commercial coastal fishery. *Hydrobiologia* 465:95-102.
- Barón, P.J., Real, L.E., Ciocco, N.F., Re, M.E., 2004. Morphometry, growth and reproduction of an Atlantic population of the razor clam *Ensis macha* (Molina, 1782). *Sci. Mar.* 68:211-217.
- Basuyaux, O., Blin, J.L., 1998. Use of maize as a food source for sea urchins in a recirculating rearing system. *Aquacult. Int.* 6:233-247.
- Bayne, B.L., 1976. Aspects of reproduction in bivalve molluscs. In: M.L. Viley (ed.) *Estuarine processes*. Academic Press, New York, USA, pp 432-448.
- Beiras, R., Pérez Camacho, A., 1994. Influence of food concentration on the physiological energetics and growth of *Ostrea edulis* larvae. *Mar. Biol.* 120:427-435.
- Beiras, R., Pérez Camacho, A., Albentosa, M., 1994. Comparison of the scope for growth with the growth performance of *Ostrea edulis* seed reared at different food concentrations in an open-flow system. *Mar. Biol.* 119:227-233.
- Beninger, P.G., Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* (L.) (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *J. Exp. Mar. Biol. Ecol.* 79:19-37.
- Berger, E., 2010. Aquaculture of *Octopus* species: present status, problems and perspectives. *Plymouth Student Scientist* 4:384-399.
- Berntsson, K.M., Jonsson, P.R., Wangberg, S.A., Carlsson, A.S., 1997. Effects of broodstock diets on fatty acid composition, survival and growth rates in larvae of the European flat oyster, *Ostrea edulis*. *Aquaculture* 154:137-151.
- BIM, 2005. European market for razor clams (*Ensis ensis* and *Ensis siliqua*). Bord Iascaigh Mhara Market development Division ed., Dublin, Ireland.
- Blanco, G.J., Villaluz, D.K., Montalban, H.R., 1951. The cultivation and biology of oysters at Bacoar Bay, Luzon. *Philipp. J. Fish.* 1:35-53.
- Boletzky, S.V., Hanlon, R.T., 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. *Mem. Natl. Mus. Victoria* 44:147-187.
- Boucaud-Camou, E., Boucher-Rodoni, R., 1983. Feeding and digestion in cephalopods. In: A.S.M. Wilbur (ed.) *The mollusca. Physiology, Part II*, vol. 5. Academic Press, London, UK.
- Boudouresque, C.F., Lemée, R., Mari, X., Meinesz, A., 1996. The invasive alga *Caulerpa taxifolia* is not a suitable diet for the sea urchin *Paracentrotus lividus*. *Aquat. Bot.* 53:245-250.
- Boudouresque, C.F., Verlaque, M., 2001. Ecology of *Paracentrotus lividus*. In: J.M. Lawrence (ed.) *Edible sea urchins: biology and ecology*. Elsevier, Amsterdam, The Netherlands, pp 177-216.
- Breber, P., 1980. Annual gonadal cycle in the carpet-shell clam *Venerupis decussata* in Venice lagoon, Italy. *Proc. Natl. Shellfish. Assoc.* 70:31-35.
- Breber, P., 1985. On-growing of the carpet shell clam (*Tapes decussatus* (L.)): Two years' experience in Venice Lagoon. *Aquaculture* 44:51-56.
- Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Mar. Biol.* 104:275-289.
- Cagnetta, P., Sublimi, A., 2000. Productive performance of the common octopus (*Octopus vulgaris* C.) when fed on a monodiet. Recent advances in Mediterranean aquaculture finfish species diversification. *Cah. Options Mediterr.* 47:331-336.
- Cano, J., Rocamora, J., 1996. Growth of the European flat oyster in the Mediterranean Sea (Murcia, SE Spain). *Aquacult. Int.* 4:67-84.
- Carlucci, R., Sassanelli, G., Matarrese, A., Giove, A., D'Onghia, G., 2010. Experimental data on growth, mortality and reproduction of *Ostrea edulis* (L., 1758) in a semi-enclosed basin of the Mediterranean Sea. *Aquaculture* 306:167-176.
- Cellario, C., Fenaux, L., 1990. *Paracentrotus lividus* (Lamarck) in culture (larval and benthic phases): parameters of growth observed during two years following metamorphosis. *Aquaculture* 84:173-188.
- Cerezo Valverde, J., García García, B., 2004. Influence of body weight and temperature on post-prandial oxygen consumption of common octopus (*Octopus vulgaris*). *Aquaculture* 233:599-613.
- Cerezo Valverde, J., García García, B., 2005. Suitable dissolved oxygen for common octopus (*Octopus vulgaris*, Cuvier, 1797) at different weights and temperatures:

- analysis of respiratory behaviour. *Aquaculture* 244:303-314.
- Cerezo Valverde, J., Gómez, E., García García, B., 2003. Resultados preliminares sobre la producción de amoníaco en el pulpo de roca (*Octopus vulgaris* Cuvier, 1797) obtenidos mediante un electrodo de ion selectivo. Libro de resúmenes. pp 305-306 in Proc. 9th Nat. Congr. Aquac., Cadiz, Spain.
- Cesari, P., Pellizzato, M., 1985. Molluschi pervenuti in Laguna di Venezia per apporti antropici volontari o casuali. Acclimatazione di *Saccostrea commercialis* (Iredale & Roughely, 1933) e di *Tapes philippinarum* (Adams & Reeves, 1850). *Boll. Malacol.* 21:237-274.
- Chessa, L.A., Paesanti, F., Pais, A., Scardi, M., Serra, S., Vitale, L., 2005. Perspectives for the development of low impact aquaculture in a Western Mediterranean lagoon: the case of the carpet clam *Tapes decussatus*. *Aquacult. Int.* 13:147-155.
- Chessa, L.A., Scardi, M., Pais, A., Serra, S., Vitale, L., Mura, F., 1998. Prove di coltivazione di *Tapes decussatus* (L.) nello stagno di Calich (Sardegna Nord-occidentale). *Biologia Marina Mediterranea* 5:1964-1972.
- Cook, E.J., Hughes, A.D., Orr, H., Kelly, M.S., Black, K.D., 2007. Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). *Aquaculture* 273:586-594.
- Cook, E.J., Kelly, M.S., 2007a. Effect of variation in the protein value of the red macroalga *Palmaria palmata* on the feeding and growth of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). *Aquaculture* 270:207-217.
- Cook, E.J., Kelly, M.S., 2007b. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. *Aquaculture* 273:573-585.
- Cook, E.J., Kelly, M.S., 2009. Co-culture of the sea urchin *Paracentrotus lividus* and the edible mussel *Mytilus edulis* L. on the west coast of Scotland, United Kingdom. *J. Shellfish Res.* 28:553-559.
- da Costa, F., Darriba, S., Martínez-Patiño, D., 2008. Embryonic and larval development of *Ensis arcuatus* (Jeffreys, 1865) (Bivalvia: Pharidae). *J. Mollus. Stud.* 74:103-109.
- da Costa, F., Darriba, S., Martínez-Patiño, D., Guerra, A., 2011a. Culture possibilities of the razor clam *Ensis arcuatus* (Pharidae: Bivalvia). *Aquac. Res.* 42:1549-1557.
- da Costa, F., Martínez-Patiño, D., 2009. Culture potential of the razor clam *Solen marginatus* (Pennant, 1777). *Aquaculture* 288:57-64.
- da Costa, F., Nóvoa, S., Ojea, J., Martínez-Patiño, D., 2011b. Changes in biochemical and fatty acid composition of the razor clam *Solen marginatus* (Solenidae: Bivalvia) during larval development. *Mar. Biol.* 158:1829-1840.
- da Silva, M.P., Fuentes, J., Villalba, A., 2005. Growth, mortality and disease susceptibility of oyster *Ostrea edulis* families obtained from brood stocks of different geographical origins, through on-growing in the Ria formosa de Arousa (Galicia, NW Spain). *Mar. Biol.* 147:965-977.
- Davis, H.C., Calabrese, A., 1969. Survival and growth of larvae of European oyster (*Ostrea edulis* L.) at different temperatures. *Biol. Bull.* 136:193-199.
- De Wolf, T., Lenzi, S., Lenzi, F., 2011. Paralarval rearing of *Octopus vulgaris* (Cuvier) in Tuscany, Italy. *Aquac. Res.* 42:1406-1414.
- Delgado, M., Pérez Camacho, A., 2002. Hermaphroditism in *Ruditapes decussatus* (L.) (Bivalvia) from the Galician coast (Spain). *Sci. Mar.* 66:183-185.
- Delgado, M., Pérez Camacho, A., 2003. A study of gonadal development in *Ruditapes decussatus* (L.) (Mollusca, Bivalvia), using image analysis techniques: influence of food ration and energy balance. *J. Shellfish Res.* 22:435-441.
- Delgado, M., Pérez-Camacho, A., 2005. Histological study of the gonadal development of *Ruditapes decussatus* (L.): (Mollusca: Bivalvia) and its relationship with available food. *Sci. Mar.* 69:87-97.
- Delgado, M., Perez-Camacho, A., 2007. Comparative study of gonadal development of *Ruditapes philippinarum* (Adams and Reeve) and *Ruditapes decussatus* (L.) (Mollusca: Bivalvia): Influence of temperature. *Sci. Mar.* 71:471-484.
- Domingues, P., Bettencourt, V., Guerra, A., 2006. Growth of *Sepia officinalis* in captivity and in nature. *Vie Milieu* 56:109-120.
- Domingues, P., Garcia, S., Garrido, D., 2010. Effects of three culture densities on growth and survival of *Octopus vulgaris* (Cuvier, 1797). *Aquacult. Int.* 18:165-174.
- Domingues, P., López, N., Muñoz, J., Maldonado, T., Gaxiola, G., Rosas, C., 2007. Effects of an artificial diet on growth and survival of the Yucatán octopus, *Octopus maya*. *Aquacult. Nutr.* 13:273-280.
- Domingues, P.M., Dimarco, F.P., Andrade, J.P., Lee, P.G., 2005. The effects of diets with amino acid supplementation on the survival, growth and body composition of the cuttlefish *Sepia officinalis*. *Aquacult. Int.* 13:423-440.
- EFSA, 2010. Scientific opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*. *European Food Safety Authority Journal* 8:1-60. Available from: <http://www.efsa.europa.eu/en/efsajournal/doc/1894.pdf>
- Enes, P., Borges, M., 2003. Evaluation of microalgae and industrial cheese whey as diets for *Tapes decussatus* (L.) seed: Effects on water quality, growth, survival, condition and filtration rate. *Aquac. Res.* 34:299-309.
- Estefanell, J., Roo, J., Guirao, R., Izquierdo, M., Socorro, J., 2012. Benthic cages versus floating cages in *Octopus vulgaris*: Biological performance and biochemical composition feeding on Boops boops discarded from fish farms. *Aquacult. Eng.* 49:46-52.
- European Commission, 2004. Commission Decision of 29 April 2004 concerning laying down specific hygiene rules for on the hygiene of foodstuffs, 04/853/EC. In: *Official Journal, L* 39, 30/04/2004.
- European Commission, 2005. Commission Decision of 15 November 2005 concerning microbiological criteria for foodstuffs, 05/2073/EC. In: *Official Journal, L* 338, 22/12/2005.
- Fabbrocini, A., Volpe, M.G., Di Stasio, M., D'Adamo, R., Maurizio, D., Coccia, E., Paolucci, M., 2011. Agar-based pellets as feed for sea urchins (*Paracentrotus lividus*): rheological behaviour, digestive enzymes and gonad growth. *Aquac. Res.* 43:321-331.
- Fahy, E., Gaffney, J., 2001. Growth statistics of an exploited razor clam (*Ensis siliqua*) bed at Gormanstown, Co Meath, Ireland. *Hydrobiologia* 465:139-151.
- FAO, 2004. Hatchery culture of bivalves. A practical manual. FAO Fisheries Technical Paper, No. 471. FAO ed., Roma, Italy. Available from: <ftp://ftp.fao.org/docrep/fao/007/y5720e/y5720e00.pdf>
- FAO, 2010. The state of world fisheries and aquaculture 2010. FAO ed., Roma, Italy. Available from: <http://www.fao.org/docrep/013/i1820e/i1820e00.htm>
- FAO, 2011. Global datasets, FishStat Plus - Universal software for fishery statistical time series. FAO ed., Roma, Italy. Available from: <http://www.fao.org/fishery/statistics/software/fishstat/en>
- Fernandez, C., 1997. Effect of diet on the biochemical composition of *Paracentrotus lividus* (Echinodermata: Echinoidea)

- under natural and rearing conditions (Effect of diet on biochemical composition of urchins). *Comp. Biochem. Phys. A* 118:1377-1384.
- Fernandez, C., Boudouresque, C.F., 2000. Nutrition of the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different artificial food. *Mar. Ecol.-Prog. Ser.* 204:131-141.
- Fernandez, C., Pergent, G., 1998. Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin *Paracentrotus lividus*. *J. Shellfish Res.* 17:1571-1581.
- Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 1998. Effect of microalgal diets and commercial wheat-germ flours on the lipid profile of *Ruditapes decussatus* spat. *Comp. Biochem. Phys. A* 119:369-377.
- Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 1999. Lipid profile and growth of the clam spat, *Ruditapes decussatus* (L), fed with microalgal diets and cornstarch. *Comp. Biochem. Phys. B* 124:309-318.
- Ferreiro, M.J., Pérez-Camacho, A., Labarta, U., Beiras, R., Planas, M., Fernández-Reiriz, M.J., 1990. Changes in the biochemical composition of *Ostrea edulis* larvae fed on different food regimes. *Mar. Biol.* 106:395-401.
- Frantzis, A., Grémare, A., 1992. Ingestion, absorption, and growth rates of *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different macrophytes. *Mar. Ecol.-Prog. Ser.* 95:169-183.
- Froglia, C., 1975. Osservazioni sull'accrescimento di *Chamelea gallina* (L.) ed *Ensis minor* (Chenu) nel medio Adriatico. *Quad. Lab. Tecnol. Pesca* 2:37-48.
- García, S., Domingues, P., Navarro, J.C., Hachero, I., Garrido, D., Rosas, C., 2011. Growth, partial energy balance, mantle and digestive gland lipid composition of *Octopus vulgaris* (Cuvier, 1797) fed with two artificial diets. *Aquacult. Nutr.* 17:174-187.
- García García, J., García García, B., 2011. Econometric model of viability/profitability of octopus (*Octopus vulgaris*) on-growing in sea cages. *Aquacult. Int.* 19:1177-1191.
- García García, J., Rodríguez-González, L.M., García García, B., 2004. Cost analysis of octopus on-growing installation in Galicia. *Span. J. Agric. Res.* 2:531-537.
- Gaspar, M.B., Monteiro, C.C., 1998. Reproductive cycles of the razor clam *Ensis siliqua* and the clam *Venus striatula* of Vilamoura, Southern Portugal. *J. Mar. Biol. Assoc. UK* 78:1247-1258.
- Gianguzza, P., Chiantore, M., Bonaviri, C., Cattaneo Vietti, R., Viellini, I., Riggio, S., 2006. The effects of recreational *Paracentrotus lividus* fishing on distribution patterns of sea urchins at Ustica Island MPA (Western Mediterranean, Italy). *Fish. Res.* 66:37-44.
- González, R., González, G., 1985. Experiencia sobre cultivo en batea de la ostra plana, *Ostrea edulis*, en la Ría de Arosa (Galicia). *Bol. Inst. Esp. Oceanogr.* 2:9-16.
- Grosjean, P., Spirlet, C., Gosselin, P., Vaitilingon, D., Jangoux, M., 1998. Land-based closed cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): a long-term experiment at a pilot scale. *J. Shellfish Res.* 17:1523-1531.
- Grosjean, P., Spirlet, C., Jangoux, M., 1996. Experimental study of growth in the echinoid *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *J. Exp. Mar. Biol. Ecol.* 201:173-184.
- Guerra Díaz, A., Lodeiros Seijo, C., Gaspar, M.B., da Costa González, F., 2011. Razor clams: biology, aquaculture and fisheries. *Consellería do Mar, Xunta de Galicia ed., Santiago de Compostela, Spain. Available from: [ftp://ftp.cimacoron.org/razor\\_clams\\_2011.pdf](ftp://ftp.cimacoron.org/razor_clams_2011.pdf)*
- Guidetti, P., Terlizzi, A., Boero, F., 2004. Effects of the edible sea urchin, *Paracentrotus lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). *Fish. Res.* 66:287-297.
- Hamazaki, H., Fukunaga, K., Yoshida, Y., Maruyama, K., 1991. Effects of marine microalgae *Nannochloropsis* sp. on survival and growth on rearing pelagic paralarvae of *Octopus vulgaris*, and results of mass culture in the tank of 20 metric tons. *Saibai Giken* 19:75-84.
- Hurtado, J.L., Borderías, J., Montero, P., An, H., 1999. Characterization of proteolytic activity in octopus (*Octopus vulgaris*) arm muscle. *J. Food Biochem.* 23:469-483.
- Hurtado, J.L., Montero, P., Borderías, J., 2001a. Behavior of octopus muscle (*Octopus vulgaris*) under a process of pressure-time-temperature combinations. *Food Sci. Technol. Int.* 7:259-267.
- Hurtado, J.L., Montero, P., Borderías, J., 2001b. Chilled storage of pressurized octopus (*Octopus vulgaris*) muscle. *J. Food Sci.* 66:400-406.
- Hurtado, J.L., Montero, P., Borderías, J., Solas, M.T., 2001c. High-pressure/temperature treatment effect on the characteristic of octopus (*Octopus vulgaris*) arm muscle. *Eur. Food Res. Technol.* 213:22-29.
- Ieropoli, S., Masullo, P., Do Espirito Santo, M., Sansone, G., 2005. Adattamento dell'ostrica *Ostrea edulis* in ambiente confinato e condizionato. *Biologia Marina Mediterranea* 12:191-194.
- Iglesias, J., Fuentes, L., Sánchez, J., Otero, J.J., Moxica, C., Lago, M.J., 2006. First feeding of *Octopus vulgaris* Cuvier, 1797 paralarvae using *Artemia*: effect of prey size, prey density and feeding frequency. *Aquaculture* 261:817-822.
- Iglesias, J., Otero, J.J., Moxica, C., Fuentes, L., Sánchez, F.J., 2002. Paralarvae culture of octopus (*Octopus vulgaris*, Cuvier) using *Artemia* and crab zoeae and first data on juvenile growth up to eight months of age. *Proc. Eur. Conf. Aquac., Trieste, Italy*, 32:268-269.
- Iglesias, J., Otero, J.J., Moxica, C., Fuentes, L., Sánchez, F.J., 2004. The completed life cycle of the octopus (*Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using *Artemia* and zoeae, and first data on juvenile growth up to 8 months of age. *Aquacult. Int.* 12:481-487.
- Iglesias, J., Sánchez, F.J., Bersano, J.G.F., Carrasco, J.F., Dhont, J., Fuentes, L., Linares, F., Muñoz, J.L., Okumura, S., Roo, J., van der Meeren, T., Vidal, E.A.G., Villanueva, R., 2007. Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. *Aquaculture* 266:1-15.
- Iglesias, J., Sánchez, F.J., Otero, J.J., 1997. Primeras experiencias sobre el cultivo integral del pulpo (*Octopus vulgaris* Cuvier) en el I.E.O. pp 221-226 in *Proc. 6th Nat. Congr. Aquac., Cartagena, Spain*.
- Iglesias, J., Sánchez, F.J., Otero, J.J., Moxica, C., 2000. Culture of octopus (*Octopus vulgaris*, Cuvier). Present knowledge, problems and perspectives. Recent advances in Mediterranean aquaculture finfish species diversification. *Cah. Options Méditerran.* 47:313-321.
- Imamura, S., 1990. Larval rearing of octopus (*Octopus vulgaris* Cuvier). The progress of technological development and some problems remained. *Collect. Breed.* 52:339-343.
- Itami, K., Izawa, Y., Maeda, S., Nakay, K., 1963. Notes on the laboratory culture of octopus larvae. *Bull. Jpn. Soc. Sci. Fish.* 29:514-520.
- Jara-Jara, R., Pazos, A.J., Abad, M., García-Martín, L.O., Sanchez, J.L., 1997. Growth of clam seed (*Ruditapes decussatus*) reared in the wastewater effluent from a fish farm in Galicia (N.W. Spain). *Aquaculture* 158:247-262.
- Jaziri, H., 1990. Variations genetiques et structuration biogeographique chez un bivalve



- marin: l'huitre plate *Ostrea edulis* L. Degree Diss., Université de Montpellier II, Montpellier, France.
- Kelly, M.S., Chamberlain, J., 2010. Recent advances in sea-urchin aquaculture and enhancement in Scotland and Ireland. *Bull. Aquacul. Assoc. Canada* 108:23-29.
- Korringa, P., 1941. Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *Ostrea edulis* L. *Arch. Neerl. Zool.* 5:1-249.
- Korringa, P., 1952. Recent advances in oyster biology. *Q. Rev. Biol.* 27:339-365.
- Kreuzer, R., 1984. Cephalopods: handling, processing and products. FAO Fish. Tech. Pap. No. 254, Food and Agriculture Organization of the United Nations ed., Roma, Italy.
- Lahbib, Y., Abidli, S., Trigui El Menif, N., 2008. Croissance des juvéniles chez *Hexaplex trunculus* (Gastropoda: Muricidae) réalisée au laboratoire. *B. Soc. Zool. Fr.* 133: 141-148.
- Laing, I., Millican, P.F., 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. *Aquaculture* 54:245-262.
- Laing, I., Walker, P., Areal, F., 2005. A feasibility study of native oyster (*Ostrea edulis*) stock regeneration in the United Kingdom. CEFAS ed., Lowestoft, UK.
- Lamela, T., Otero, A., Arredondo-Vega, B.O., Patiño, M., Fábregas, J., 1996. Artificial diets as substitutes of microalgae for the culture of clam spat (*Tapes decussatus*). *J. Mar. Biotechnol.* 3:278-282.
- Laruelle, F., Guillou, J., Paulet, Y.M., 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *Ruditapes philipinarum* on intertidal flats in Brittany. *J. Mar. Biol. Assoc. UK* 74:351-366.
- Le Gall, P., 1989. L'échinoculture. In: G. Barnabé (ed.) *Aquaculture* Vol. 1. Lavoisier, Paris, France, pp 467-491.
- Lee, P.G., 1994. Nutrition of cephalopods: fuelling the system. *Mar. Behav. Physiol.* 25:35-51.
- Lemée, R., Boudouresque, C.F., Gobert, J., Malestroit, P., Mari, X., Meinesz, A., Menager, V., Ruitton, S., 1996. Feeding behaviour of *Paracentrotus lividus* in the presence of *Caulerpa taxifolia* introduced in the Mediterranean Sea. *Oceanol. Acta* 19:245-253.
- Libertini, A., Boato, A., Panozzo, M., Fogato, V., 1996. Karyotype and genome size in some species of *Mytilidae* (*Bivalvia*, *Mollusca*). *La Kromosomo* 11 82:2819-2827.
- Loosanoff, V., Davies, H.C., 1963. Rearing of bivalve larvae. *Adv. Mar. Biol.* 1:1-136.
- Lozano, J., Galera, J., Lopez, S., Turon, X., Palacin, C., Morera, G., 1995. Biological cycles and recruitment of *Paracentrotus lividus* (*Echinodermata*: *Echinoidea*) in two contrasting habitats. *Mar. Ecol. Prog. Ser.* 122:179-191.
- Lubet, P., 1959. Recherches sur le cycle sexuel et l'émission des gamètes chez les *Mytilidae* et les *Pectinidae* (*Mollusques bivalves*). *Rev. Trav. Inst. Pêches Marit.* 23:387-548.
- Lubrano Lavadera, S., Fabbrocini, A., Rispoli, S., Sansone, G., 1999. Prove di crescita di *Ostrea edulis* presso allevamento ittico "off-shore". *Biologia Marina Mediterranea* 6:325-327.
- Lucas, A., 1975. Sex differentiation and juvenile sexuality in bivalves molluscs. *Pubbl. Staz. Zool. Napoli* 39(Suppl.):532-541.
- Luis, O., Delgado, F., Gago, J., 2005. Year-round captive spawning performance of the sea urchin *Paracentrotus lividus*: relevance for the use of its larvae as live feed. *Aquat. Living Resour.* 18:45-54.
- Mangold, K., Boletzky, S.V., 1973. New data on reproductive biology and growth of *Octopus vulgaris*. *Mar. Biol.* 19:7-12.
- Mann, R., 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *J. Mar. Biol. Assoc. UK* 59:95-110.
- Marcaillou, C., Haure, J., Mondeguer, F., Courcoux, A., Dupuy, B., Pénisson, C., 2010. Effect of food supply on the detoxification in the blue mussel, *Mytilus edulis*, contaminated by diarrhetic shellfish toxins. *Aquat. Living Resour.* 23:255-266.
- Martínez-Patiño, D., da Costa, F., Cromie, A., Nóvoa, S., Ojea, J., Werner, A., Browne, L., Fernández-Tajes, J., Méndez, J., Gaspar, M., Constantino, R., Roberts, D., McDonough, N., 2007. Desarrollo de la acuicultura de navajas y longueirones: cultivo en criadero. pp 611-614 in *Proc. 11th Nat. Congr. Aquac.*, Vigo, Spain.
- Martínez-Pita, I., Hachero-Cruzado, I., Sánchez-Lazo, C., Moreno, O., 2012. Effect of diet on the lipid composition of the commercial clam *Donax trunculus* (*Mollusca*: *Bivalvia*): sex-related differences. *Aquac. Res.* 43:1134-1144.
- Martínez-Pita, I., Sánchez-Lazo, C., Prieto, E., Moreno, O., 2011. The effect of diet on gonadal development of the smooth venus clam *Callista chione* (*Mollusca*: *Bivalvia*). *J. Shellfish Res.* 30:295-301.
- Mather, J.A., 2008. Cephalopod consciousness: behavioural evidence. *Conscious. Cogn.* 17:37-48.
- Matias, D., Joaquim, S., Leitão, A., 2009. Effect of geographic origin and timing of brood-stock collection on conditioning, spawning success and larval viability of *Ruditapes decussatus* (Linnaeus, 1758). *Aquacult. Int.* 17:257-271.
- Matias, D., Joaquim, S., Ramos, M., Sobral, P., Leitão, A., 2011. Biochemical compounds' dynamics during larval development of the carpet-shell clam *Ruditapes decussatus* (Linnaeus, 1758): Effects of mono-specific diets and starvation. *Helgolander Mar. Res.* 65:369-379.
- Medhioub, W., Guéguen, M., Lassus, P., Bardouil, M., Truquet, P., Sibat, M., Medhioub, N., Soudant, P., Kraiem, M., Amzil, Z., 2010. Detoxification enhancement in the gymnodimine-contaminated grooved carpet shell, *Ruditapes decussatus* (Linnaeus). *Harmful Algae* 9:200-207.
- Mendes, R., Alves Silva, H., Anacleto, P., Cardoso, C., 2011. Effect of CO<sub>2</sub> dissolution on the shelf life of ready-to-eat *Octopus vulgaris*. *Innov. Food Sci. Emerg. Technol.* 12:551-561.
- Millican, P.F., Helm, M.M., 1994. Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. *Aquaculture* 123:83-94.
- Moxica, C., Linares, F., Otero, J.J., Iglesias, J., Sánchez, F.J., 2002. Cultivo intensivo de paralarvas de pulpo, *Octopus vulgaris* Cuvier, 1797, en tanques de 9 m<sup>3</sup>. *Bol. Inst. Esp. Oceanogr.* 18:31-36.
- Navarro, J.C., Villanueva, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* 183:161-177.
- Navarro, J.C., Villanueva, R., 2003. The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. *Aquaculture* 219:613-631.
- Newell, R.I.E., Hilbish, T.J., Koehn, R.K., Newell, C.J., 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (*Bivalvia*, *Mytilidae*) from localities on the east coast of the United States. *Biol. Bull.* 162:299-310.
- O'Dor, R.K., Mangold, K., Boucher-Rodoni, R., Wells, M.J., Wells, J., 1983. Nutrient absorption, storage and remobilization in *Octopus vulgaris*. *Mar. Behav. Physiol.* 11:239-258.
- O'Dor, R.K., Wells, M.J., 1978. Reproduction versus somatic growth: hormonal control in *Octopus vulgaris*. *J. Exp. Biol.* 77:529-540.
- Ojea, J., Pazos, A.J., Martínez, D., Novoa, S., García-Martínez, P., Sánchez, J.L., Abad, M., 2008. Effects of temperature regime on

- broodstock conditioning of *Ruditapes decussatus*. *J. Shellfish Res.* 27:1093-1100.
- Ojea, J., Pazos, A.J., Martínez, D., Novoa, S., Sanchez, J.L., Abad, M., 2004. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. *Aquaculture* 238:451-468.
- Otero, J.J., Moxica, C., Sánchez, F.J., Iglesias, J., 1999. Engorde de pulpo (*Octopus vulgaris* Cuvier) a diferentes densidades de estabulación. pp 180-184 in Proc. 7th Nat. Congr. Aquac., Las Palmas de Gran Canaria, Spain.
- Ozogul, Y., Duysak, O., Ozogul, F., Özkütük, A.S., Türeli, C., 2008. Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. *Food Chem.* 108:847-852.
- Paesanti, F., Pellizzato, M., 2000. Tapes philippinarum. Manuale sulla vongola verace d'allevamento. ESAV ed., Padova, Italy. Available from: [http://www.pescapolesine.it/sito/pagine/dynamic.php?table\\_name=pesca\\_carta\\_ittica\\_vongola](http://www.pescapolesine.it/sito/pagine/dynamic.php?table_name=pesca_carta_ittica_vongola)
- Pais, A., Chessa, L.A., Serra, S., Meloni, G., Ruiú, A., Manunza, B., 2006a. Morphometric relationships and annual gonad index of the edible sea urchin *Paracentrotus lividus* from North western Sardinia. *Biologia Marina Mediterranea* 13:202-203.
- Pais, A., Chessa, L.A., Serra, S., Ruiú, A., 2006b. An alternative suspended culture method for the Mediterranean carpet clam, *Tapes decussatus* (L.), in the Calich lagoon (North western Sardinia). *Biologia Marina Mediterranea* 13:134-135.
- Pais, A., Chessa, L.A., Serra, S., Ruiú, A., Meloni, G., 2007. Suspended culture of *Ostrea edulis* (Bivalvia, Ostreidae) in the Calich lagoon (North western Sardinia, Italy): preliminary results. *Ital. J. Anim. Sci.* 6(Suppl.1):810 (abstr.).
- Pais, A., Chessa, L.A., Serra, S., Ruiú, A., Meloni, G., Donno, Y., 2007. The impact of commercial and recreational harvesting for *Paracentrotus lividus* on shallow rocky reef sea urchin communities in North-western Sardinia, Italy. *Estuar. Coast. Shelf S.* 73:589-597.
- Pais, A., Saba, S., Campus, P., Gorla, A., 2012a. Sopravvivenza e crescita dell'ostrea piatta (*Ostrea edulis* Linnaeus, 1758) nello Stagno di San Teodoro (Sardegna nord orientale). pp. 158-159 in Proc. 43rd Nat. Congr. SIBM, Marina di Camerota (SA), Italy.
- Pais, A., Serra, S., Meloni, G., Saba, S., Ceccherelli, G., 2012b. Harvesting effects on *Paracentrotus lividus* population structure: a case study from Northwestern Sardinia, Italy before and after the fishing season. *J. Coast. Res.* 28:570-575.
- Pantazis, P.A., 2009. The culture potential of *Paracentrotus lividus* (Lamarck 1816) in Greece: a preliminary report. *Aquacult. Int.* 17:545-552.
- Pastore, M., Prato, E., Palagiano, B., 1996. Allevamento in sospensione di *Tapes decussatus* (L.) (Mollusca, Veneridae) nel Mar Piccolo di Taranto. *Quaderni dell'Istituto di idrobiologia e acquacoltura "G. Brunelli"* 15:19-40.
- Pellizzato, M., Da Ros, L., 1985. Allevamento sperimentale di *Ostrea edulis* L. e *Crassostrea gigas* (Thunberg) in Laguna di Venezia. *Oebalia* 11:891-893.
- Pellizzato, M., Galvan, T., Penzo, P., 2005. Prospettive di ostricoltura in alto Adriatico. *Biologia Marina Mediterranea* 12:223-226.
- Pellizzato, M., Libralato, M., Nesto, N., 1998. Molluschicoltura in biotopi marginali della laguna di Venezia. *Biologia Marina Mediterranea* 5:1917-1926.
- Pérez Camacho, A., Albentosa, M., Fernández-Reiriz, M.J., Labarta, U., 1998. Effect of microalgal and inert (cornmeal and cornstarch) diets on growth performance and biochemical composition of *Ruditapes decussatus* seed. *Aquaculture* 160:89-102.
- Pérez Camacho, A., Beiras, R., Albentosa, M., 1994. Effects of algal food concentration and body size on the ingestion rates of *Ruditapes decussatus* (Bivalvia) veliger larvae. *Mar. Ecol. Prog. Ser.* 115:87-92.
- Pérez Camacho, A., Roman, G., 1985. Cultivo en batea de semilla de ostra *Ostrea edulis* en la Ria de Arosa. *Bol. Inst. Esp. Oceanogr.* 2:1-8.
- Pérez Camacho, A., Salinas, J.M., Delgado, M., Fuertes, C., 2007. Use of single cell detritus (SCD) produced from laminaria saccharina in the feeding of the clam *Ruditapes decussatus* (Linnaeus, 1758). *Aquaculture* 266:211-218.
- Perez-Larruscain, J., Delgado, M., Gairin, J.I., 2011. Larval growth and development of the smooth clam *Callista chione* (Mollusca: Bivalvia) under laboratory conditions. *Cienc. Mar.* 37:271-277.
- Piccinetti, C., 2011. Studio e messa a punto della tecnica di riproduzione del tartufo di mare "Venus verrucosa" e semina sperimentale di novellame in aree di pesca programmate ed autogestite. In: *La ricerca scientifica a supporto della pesca e dell'acquacoltura*. Consorzio UNIMAR ed., Roma, Italy, pp 126-128.
- Pogoda, B., Buck, B.H., Hagen, W., 2011. Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany). *Aquaculture* 319:484-492.
- Prioli, G., 2004. Shellfish farming: technologies and production. *Vet. Res. Commun.* 28:51-56.
- Prioli, G., 2008. La molluschicoltura in Italia. In: A. Lovatelli, A. Fariás and I. Uriarte (eds.) *Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura. Factores que afectan su sustentabilidad en América Latina*. FAO ed., Roma, Italy, pp 159-176.
- Prioli, G., Mietti, N., Malorgio, A.G., Maffei, M., 1998. Messa a punto di nuove tecnologie di allevamento di *Mytilus galloprovincialis* e *Modiolus barbatus*. *Biologia Marina Mediterranea* 5:1857-1866.
- Quayle, D.B., 1980. Tropical oysters: culture and methods. International Development Research Center ed., Ottawa, Canada.
- Ramón, M., Flos, R., 2001. First trials to cultivate the muricid gastropod *Bolinus brandaris* (Linnaeus). *Eur. Aquac. Soc. Spec. Publ.* 29:219-220.
- Rasmussen, M.R., McLean, E., 2004. Comparison of two different methods for evaluating the hydrodynamic performance of an industrial-scale fish-rearing unit. *Aquaculture* 242:397-416.
- Régis, M.B., Pérès, J.M., Gras, G., 1986. Données préliminaires sur l'exploitation de la ressource *Paracentrotus lividus* dans le quartier maritime de Marseille. *Vie Marine* 7:41-60.
- Robert, R., 2009. Review on molluscs hatchery nursery development in Europe. pp 1-12 in Proc. Symp. EAS, EUROSHELL Session, Trondheim, Norway.
- Rodríguez-Moscó, E., Arnaiz, R., 1998. Gametogenesis and energy storage in a population of the grooved carpet-shell clam, *Tapes decussatus* (Linné, 1787), in northwest Spain. *Aquaculture* 162:125-139.
- Rodstrom, E.M., Jonsson, P., 2000. Survival and feeding activities of oyster spat (*Ostrea edulis* L.) as a function of temperature and salinity with implications for culture policies on the Swedish west coast. *J. Shellfish Res.* 19:799-808.
- Rosas, C., Cuzon, G., Pascual, C., Gaxiola, G., López, N., Maldonado, T., Domingues, P., 2007. Energy balance of *Octopus maya* fed crab and artificial diet. *Mar. Biol.* 152:371-378.
- Rossi, R., 2011. Studi sulla riproduzione controllata di molluschi: il dattero bianco,

- Pholas dactylus ed il tartufo di mare Venus verrucosa; e sui fattori neuroendocrini coinvolti nella loro riproduzione e sviluppo; consolidamento delle pratiche di produzione di seme di Modiolus barbatus. In: La ricerca scientifica a supporto della pesca e dell'acquacoltura. Consorzio UNIMAR ed., Roma, Italy, pp 114-116.
- Rossi, R., Paesanti, F., Turolla, E., 1998. Prove di riproduzione controllata di molluschi bivalvi. *Biologia Marina Mediterranea* 5:1888-1895.
- Ruiz-Azcona, P., Rodríguez-Sierra, R., Martín, J.B., 1996. Culture of coquina clam, *Donax trunculus*, larvae. *Aquaculture* 139:151-155.
- Sánchez, F.J., Fuentes, L., Otero, J.J., Lago, M.J., Linares, F., Pazos, G., Iglesias, J., 2011. Effect of tank volume on the growth and survival of reared *Octopus vulgaris* paralarvae. *Acquac. Res.* DOI: 10.1111/j.1365-2109.2011.03049.x
- Sanchez-Espana, A.I., Martinez-Pita, I., Garcia, F.J., 2004. Gonadal growth and reproduction in the commercial sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: Echinoidea) from southern Spain. *Hydrobiologia* 519:61-72.
- Sastry, A.N., 1975. Physiology and ecology of reproduction in marine invertebrates. In: F.J. Vernberg (ed.) *Physiological ecology of estuarine organisms*. University of South Carolina Press, Columbia, SC, USA, pp 279-299.
- Schuenhoff, A., Shpigel, M., Lupatsch, I., Ashkenazi, A., Msuya, F.E., Neori, A., 2003. A semi-recirculating, integrated system for the culture of fish and seaweed. *Aquaculture* 221:167-181.
- Serdar, S., Lök, A., 2009. Gametogenic cycle and biochemical composition of the transplanted carpet shell clam *Tapes decussatus*, Linnaeus 1758 in Sufa (Homa) Lagoon, Izmir, Turkey. *Aquaculture* 293:81-88.
- Serdar, S., Lök, A., Köse, A., Yildiz, H., Acarli, S., Goulletquer, P., 2007. Growth and survival rates of carpet shell clam (*Tapes decussatus* Linnaeus, 1758) using various culture methods in Sufa (Homa) Lagoon, Izmir, Turkey. *Aquacult. Eng.* 37:89-99.
- Serratore, P., 2011. Innovazione dei sistemi di allevamento dei mitili per la prevenzione dei fenomeni di contaminazione da tossine marine. In: La ricerca scientifica a supporto della pesca e dell'acquacoltura. Consorzio UNIMAR ed., Roma, Italy, pp 374-378.
- Shafee, M.S., Daoudi, M., 1991. Gametogenesis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from the Atlantic coast of Morocco. *Aquacult. Fish. Manag.* 22:203-216.
- Shpigel, M., McBride, S.C., Marciano, S., Lupatsch, I., 2004. The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. *Aquaculture* 232:343-355.
- Shpigel, M., McBride, S.C., Marciano, S., Ron, S., Ben-Amotz, A., 2005. Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. *Aquaculture* 245:101-109.
- Sinanoglou, V.J., Batrinou, A., Konteles, S., Sfimos, K., 2007. Microbial population, physicochemical quality, and allergenicity of molluscs and shrimp treated with Cobalt-60 gamma radiation. *J. Food Protect.* 70:958-966.
- Spencer, G.E., Gough, C.J., 1978. The growth and survival of experimental batches of hatchery-reared spat of *Ostrea edulis* L. and *Crassostrea gigas* Thunberg, using different methods of tray cultivation. *Aquaculture* 13:293-312.
- Spirlet, C., Grosjean, P., Jangoux, M., 1998a. Optimizing food distribution in closed-circuit cultivation of edible sea urchins (*Paracentrotus lividus*: Echinoidea). *Aquat. Living Resour.* 11:273-277.
- Spirlet, C., Grosjean, P., Jangoux, M., 1998b. Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. *Invertebr. Reprod. Dev.* 34:69-81.
- Spirlet, C., Grosjean, P., Jangoux, M., 2000. Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, *Paracentrotus lividus* (Lamarck) (Echinodermata). *Aquaculture* 185:85-99.
- Spirlet, C., Grosjean, P., Jangoux, M., 2001. Cultivation of *Paracentrotus lividus* (Echinodermata: Echinoidea) on extruded feeds: digestive efficiency, somatic and gonadal growth *Aquacult. Nutr.* 7:91-99.
- Tortonese, E., 1965. Echinodermata. *Fauna d'Italia Vol. VI*. Calderini ed., Bologna, Italy.
- Tuck, I.D., Bailey, N., Harding, M., Sangster, G., Howell, T., Graham, N., Breen, M., 2000. The impact of water jet dredging for razor clams, *Ensis* spp., in a shallow sandy subtidal environment. *J. Sea Res.* 43:65-81.
- Turolla, E., 1998. Molluschicoltura: nuove prospettive. *Laguna* 3:10-15.
- Turolla, E., 2008. La venericoltura in Italia. In: A. Lovatelli, A. Fariás and I. Uriarte (eds.) *Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura. Factores que afectan su sustentabilidad en América Latina*. FAO ed., Roma, Italy, pp 177-188.
- Turolla, E., Castaldelli, G., Barbin, L., Rossi, R., 2002. Manuale guida alla riproduzione controllata di *Modiolus barbatus*. Technical Report. Dip. Biologia. Università di Ferrara ed., Ferrara, Italy.
- Turolla, E., Rossi, R., 1999. Controlled reproduction of bearded horse mussel (*Modiolus barbatus*). Page 89 in Proc. Conf. Towards the year 2000, Verona, Italy.
- Urrutia, M.B., Ibarrola, I., Iglesias, J.I.P., Navarro, E., 1999. Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdaibai Estuary (Basque Country, N. Spain). *J. Sea Res.* 42:35-48.
- Utting, S.D., 1988. Growth and survival of hatchery-reared *Ostrea edulis* in relation to environmental conditions at the on-growing site. *Aquaculture* 69:27-38.
- Vasconcelos, P., Gaspar, M.B., Joaquim, S., Matias, D., Castro, M., 2004. Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatching and juvenile growth rates. *Int. J. Inver. Rep. Dev.* 46:125-138.
- Vasconcelos, P., Pereira, A.M., Constantino, R., Barroso, C.M., Gaspar, M.B., 2012. Growth of the purple dye murex, *Bolinus brandaris* (Gastropoda: Muricidae), marked and released in a semi-intensive fish culture earthen pond. *Sci Mar.* 76:67-78.
- Vaz-Pires, P., Seixas, P., Barbosa, A., 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* 238:221-238.
- Vercaemer, B., Spence, K., Herbinger, C., Lapegue, S., Kenchington, E., 2006. Genetic diversity of the European oyster (*Ostrea edulis*) in Nova Scotia: assessment and implications for broodstock management. *J. Shellfish Res.* 25:543-551.
- Viera, M.P., Courtois de Vico, G., Gómez-Pinchetti, J.L., Bilbao, A., Fernandez-Palacios, H., Izquierdo, M.S., 2011. Comparative performances of juvenile abalone (*Haliotis tuberculata* coccinea Reeve) fed enriched vs non-enriched macroalgae: effect on growth and body composition. *Aquaculture* 319:423-429.
- Villanueva, R., 1994. Decapod crab zoeae as food for rearing cephalopod paralarvae. *Aquaculture* 128:143-152.
- Villanueva, R., 1995. Experimental rearing and growth of planktonic *Octopus vulgaris*



- from hatching to settlement. *Can. J. Fish. Aquat. Sci.* 52:2639-2650.
- Villanueva, R., Bustamante, P., 2006. Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae. *Aquaculture* 261:225-240.
- Villanueva, R., Escudero, J.M., Deulofeu, R., Bozzano, A., Casoliva, C., 2009. Vitamin A and E content in early stages of cephalopods and their dietary effects in *Octopus vulgaris* paralarvae. *Aquaculture* 286:277-282.
- Villanueva, R., Koueta, N., Riba, J., Boucaud-Camou, E., 2002. Growth and proteolytic activity of *Octopus vulgaris* paralarvae with different food rations during first-feeding, using *Artemia* nauplii and compound diets. *Aquaculture* 205:269-286.
- Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. *Oceanogr. Mar. Biol.* 46:105-202.
- Villanueva, R., Nozais, C., Boletzky, S.V., 1995. The planktonic life of octopuses. *Nature* 377:107 (abstr.).
- Villanueva, R., Nozais, C., Boletzky, S.V., 1996. Swimming behaviour and food searching in planktonic *Octopus vulgaris* Cuvier from hatching to settlement. *J. Exp. Mar. Biol. Ecol.* 208:169-184.
- Villanueva, R., Riba, J., Ruiz-Capillas, C., González, A.V., Baeta, M., 2004. Amino acid composition of early stages of cephalopods and effect of amino acid dietary treatments on *Octopus vulgaris* paralarvae. *Aquaculture* 242:455-478.
- Walne, P.R., 1976. Experiments on the culture in the sea of the Butterfish *Venerupis decussata* L. *Aquaculture* 8:371-381.
- Walne, P.R., 1979. *Culture of Bivalve Molluscs: 50 years' experience at Conwy*. Fishing News Books, Farnham, UK.
- Wilson, J.H., 1987. Environmental parameters controlling growth of *Ostrea edulis* L. and *Pecten maximus* L. in suspended culture. *Aquaculture* 64:119-131.
- Wilson, J.H., Simons, J., 1985. Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. *Aquaculture* 46:307-321.
- Xie, Q., Burnell, G.M., 1994. A comparative study of the gametogenic cycles of the clams *Tapes philippinarum* (A. Adams & Reeve 1850) and *Tapes decussatus* (Linnaeus) on the south coast of Ireland. *J. Shellfish Res.* 13:467-472.
- Yildiz, H., Berber, S., Acarli, S., Vural, P., 2011. Seasonal variation in the condition index, meat yield and biochemical composition of the flat oyster *Ostrea edulis* (Linnaeus, 1758) from the Dardanelles, Turkey. *Ital. J. Anim. Sci.* 10:22-26.
- Yokota, Y., Matranga, V., Smolenicka, Z., 2002. The sea urchin: from basic biology to aquaculture. Swets & Zeitlinger, Lisse, The Netherlands.
- Zentilin, A., Pellizzato, M., Rossetti, E., Turolla, E., 2008. La venericoltura in Italia a 25 anni dal suo esordio. *Il Pesce* 3:31-40.
- Zlatanov, S., Laskaridis, K., Feist, C., Sagredos, A., 2006. Proximate composition, fatty acid analysis and protein digestibility-corrected amino acid score of three Mediterranean cephalopods. *Mol. Nutr. Food Res.* 50:967-970.
- Zrncić, S., Orai, D., Mihaljević, Ž., Zanella, D., 2007. Impact of varying cultivation depths on growth rate and survival of the European flat oyster *Ostrea edulis*, L. *Aquac. Res.* 38:1305-1310.